Cervico-Vaginal Fluid $\beta$-Subunit Human Chorionic Gonadotrophin for Diagnosis of Preterm Premature Rupture of Membranes

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Abstract

Objective: To determine the diagnostic value of qualitative testing of $\beta$-subunit human chorionic gonadotrophin ($\beta$-HCG) in the cervico-vaginal fluid for the diagnosis of preterm premature rupture of membranes (PPROM).

Design: A prospective, cross section, controlled study.

Setting: Departments of Obstetrics and Gynecology, Benha University Hospital and Benha Insurance Hospital, during the period from January 2009 to December 2009.

Subjects and Methods: The study was performed on two groups of pregnant women between 28-34 weeks of gestation each 50 cases. The PPRM group was pregnant with recent history of watery vaginal discharge and confirmed diagnosis of rupture of membranes evident by visualization of amniotic fluid leaking from the cervix. The control group was normal pregnant, having no complaints and attending for routine antenatal care. Three drops of the liquor from the PPRM group, or the saline washed vaginal discharge from the normal control group were aspirated and applied on the one step cassette style $\beta$-HCG urine pregnancy test (IND Diagnostic Inc-Canada). This test is sensitive at HCG level of 20mIU/ml which is higher than the mean level in the cervico-vaginal discharge of normal pregnancy; so a positive test indicated amniotic fluid and a negative test exclude amniotic fluid.

Results: The test was 90% sensitive and 98% specific in the detection of amniotic fluid and diagnosis of PPRM. The positive predictive value was 97.8%, the negative predictive value was 90.7%, and the overall accuracy of the test was 94%.

Conclusions: The qualitative $\beta$-HCG urine pregnancy test with a sensitivity of 20mIU/ml is simple and cheap test that can be used as an outpatient or a bedside test for rapid diagnosis of PPROM in the third trimester.

Key Words: PROM – Cervico – Vaginal – Chorionic – Gonadotrophin.

Introduction

PRETERM premature rupture of membranes complicates about 3% of pregnancy [1], and carries serious threatens to the mother as chorioamnionitis, preterm labor, placental abruption and post partum endometritis and to the fetus as fetal and neonatal infection, cord compression and prolapse, respiratory distress syndrome and brain damage [2]. Accurate and early diagnosis of PPROM is of great importance to avoid these complications; on the other hand, a false-positive diagnosis can lead to unnecessary intervention like administration of antibiotics and corticosteroids, hospitalization and even induction of labor [3]. Patient history and physical examination can diagnose over 90% of cases of PPROM especially when large amount of liquor is leaking, however, on many occasions the leak may be scanty or intermittent and the membranes can be ruptured without evident inspection of the amniotic fluid.

Traditional testes for diagnosis of PPROM as nitrazine, evaporation, ferning, and staining of fetal cells tests are associated with high false negative and false positive results [4]. Detection of amniotic fluid biomarkers that are present in the setting of rupture of membranes but absent or present at a very low levels in the cervico-vaginal discharge in women with intact membranes has been studied for diagnosis of PPROM with the primary goal of discovering an easy, accurate, and rapid test that enable the physician to initiate therapy without delay [5].

The list of tests used includes fetal fibronectin [6], alpha-feto-protein [7], insulin like growth factor binding protein-1 [8], prolactin [9], creatinine and urea [10], lactate [11] and placental alpha-microglobulin 1 [12]. The results of these tests have been
variable and some of them are expensive and needs sophisticated laboratory facilities.

Human chorionic gonadotrophin is a glycoprotein hormone produced in pregnancy and present at a high level in the maternal blood, urine and amniotic fluid, and is secreted by the cervical glands, so present at a very low level in the vaginal fluid [13,14]. The quantitative testing of \( \beta \)-HCG is time consuming and expensive; on the contrary, qualitative testing is simple, rapid, inexpensive, and may aid in the diagnosis of rupture of membranes [15] by using a conventional pregnancy test with a threshold value for \( \beta \)-HCG levels higher than that present in the cervico-vaginal fluid of normal pregnant women as determined by [13].

**Subjects and Methods**

The study was performed on two groups of pregnant women between 28-34 weeks of gestation each 50 cases. The PPROM group was pregnant with recent history of watery vaginal discharge and confirmed diagnosis of rupture of membranes evident by visualization of amniotic fluid leaking from the cervix. The control group was normal pregnant, having no complaints and attending for routine antenatal care. A sterile Cusco’s speculum was applied to visualize the cervix; cases with blood contamination were excluded. The posterior vaginal fornix in the control group was irrigated by 3ml saline and aspirated, and 3ml of the liquor from the PPROM group were also aspirated. Three drops were applied on the one step cassette style \( \beta \)-HCG urine pregnancy test (IND Diagnostic Inc-Canada). The test is sensitive at a threshold of 20 mIU/ml, which is higher than the mean HCG level present in the cervico-vaginal discharge at the third trimester of normal pregnancy as measured by [13]. Depending on the concentration of HCG in the specimen, positive result may be observed in as soon as 40 seconds, however, to confirm negative result, the complete reaction time of 5 minutes is required and don’t read the result after more than 30 minutes. A negative test is indicated by only one color band on the control region and excluded amniotic fluid. A positive test is indicated by distinct color band on both the control and the test. Results and indicated amniotic fluid. An invalid test is indicated by no visible band at all or one colored band appears on the control region. In the latter condition the test was repeated with a new kit.

Sensitivity, specificity, positive and negative predictive values and accuracy of the test were calculated.

**Results**

Table (1) shows non significant difference between both groups as regard maternal age, parity and residence.

Table (1): Comparison between the bio data of both groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>PPROM</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>26.3±4.5</td>
<td>26.8±4.4</td>
<td>NS</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primi</td>
<td>4 (48%)</td>
<td>22 (44%)</td>
<td>NS</td>
</tr>
<tr>
<td>Multi</td>
<td>26 (52%)</td>
<td>28 (56%)</td>
<td>NS</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>25 (50%)</td>
<td>35 (70%)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Urban</td>
<td>25 (50%)</td>
<td>15 (30%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table (2) shows that qualitative testing of \( \beta \)-HCG in the cervico-vaginal fluid for diagnosis of PPROM was 90% sensitive, 98% specific, had 97.8% positive predictive value and 90.7% negative predictive value and an overall accuracy of 94%.

Table (2): Diagnostic power of qualitative B-HCG test in the diagnosis of PPROM.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Control</th>
<th>PPROM</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCG +ve</td>
<td>1</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>-ve</td>
<td>49</td>
<td>5</td>
<td>50</td>
</tr>
</tbody>
</table>

|     | 50   | 50   | 100  |

Sensitivity: 90%
Specificity: 98%
Positive predictive value: 97.8%
Negative predictive value: 90.7%
Accuracy: 94%

**Discussion**

Anai et al. [13] reported a mean of 6.3 and a range of 0.6-62.2 mIU/ml hCG level in the cervico-vaginal discharge in normal pregnancy during the third trimester compared to 420.6 (216.3-918.3 mIU/ml) in the amniotic fluid during the same gestational age and found that at an hCG level of 50 mIU/ml, the test was 100% sensitive, 96.95% specific, 88.9% positive predictive value and 100% negative predictive value in the diagnosis of PPROM.

Mangano et al. [16] measured hCG level in the washing fluid of the posterior fornix and suggested an hCG cut-off value higher than 100 mIU/ml for diagnosis of PROM. They mentioned that there was no overlapping between the hCG levels of pregnant women without rupture of membranes.
and patients with confirmed rupture of membranes and concluded that the hCG levels in the washing fluid of the posterior vaginal fornix was a useful, very cheap and non-invasive diagnostic test of PROM.

In a study by [17] they reported that at a cut-off beta-hCG value of 65 mIU/ml, the test had 68% sensitivity, 95% specificity, 82% positive predictive value, 90% negative predictive value and 87% accuracy and concluded that vaginal washing fluid beta-hCG determination for the diagnosis of PROM is reliable, simple and rapid test.

Other investigators [18] found that the median and range of vaginal fluid $\beta$-HCG were 512.53 (26.5-3507.2 mIU/ml) in pregnant women with PPROM and reported 95.5% sensitivity, 94.7% specificity, 91.3% positive predictive value and 97.3% negative predictive value at a cut-off value of 39.8 mIU/ml for the diagnosis of PROM. The authors concluded that measurement of vaginal fluid beta-hCG is reliable, simple, and rapid test in diagnosis of PROM and can be used as an adjunctive test in equivocal cases.

In 2004 Cooper et al. [19] suggested using qualitative rather than quantitative $\beta$-HCG for diagnosis of rupture of membranes. Using a test with a sensitivity of 25 mIU/ml, they reported 79% sensitivity, 96% specificity, 95% positive predictive value and 84% negative predictive value in the diagnosis of rupture of membranes.

In the present study we used a qualitative $\beta$-HCG urine pregnancy test with a sensitivity of 20 mIU/ml. The test was 90% sensitive in the detection of the amniotic fluid in the PPRM group. There were five cases of false negative result (10%) 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.

The specificity of the test was 98%. There was one case of false positive result (2%) which may be due to high hCG level in the vaginal discharge in normal pregnant (0.6-62.2 mIU/ml) as reported by [13] which is higher than the sensitivity of the test used of 20 mIU/ml, or that microscopic contamination of vaginal discharge by maternal blood may give false positive results.

So, selection of the test is crucial as its sensitivity must be higher than the hCG level in the cervico-vaginal discharge in normal pregnancy to be a reliable indicator of amniotic fluid in cases of diagnosis of rupture of membranes.

Other investigators [20] found that vaginal fluid alpha-fetoprotein and creatinin were superior to hCG in detecting PROM, while [21] found that alpha-fetoprotein had higher diagnostic performance than prolactin or hCG in diagnosis of PPROM.

In another study [22] reported that both qualitative and quantitative assay of vaginal fluid hCG are not useful in diagnosis of PROM. This result might be due to difference in the selection criteria from our study where the authors did the test immediately pre and post rupture of fetal membranes during labor; so, microscopic blood contamination or undetected scanty amniotic fluid leak due to uterine contractions might interfere with interpretations of results.

However, in a more recent study [23] 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.

Conclusions: The qualitative $\beta$-HCG urine pregnancy test with a sensitivity of 20 mIU/ml is simple and cheap test that can be used as an outpatient or a bedside test for rapid diagnosis of PPRM in the third trimester. The higher the level of sensitivity of the test used the higher will be its specificity in the diagnosis of PPROM.

References

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