Various Biomarkers In Diagnosing Premature Rupture Of Membranes: A Cost Effective Analysis

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Citation

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Abstract
Introduction: To determine the most reliable marker amongst β human chorionic gonadotropin (βhCG), ά fetoprotein (AFP), prolactin and creatinine in vaginal washing for diagnosing premature rupture of membranes (PROM) and analysing their cost effectiveness.

Methods: 100 pregnant women between 20-40 weeks of gestation were recruited and divided into 2 groups. The study group consisted of 50 women with history of leaking per vaginum and control group comprised of 50 normal pregnant women from antenatal clinic. All women underwent speculum examination aiming to sample and assay the 4 markers. The cost effectiveness of each marker was determined. Data was analyzed by student’s t-test, receiver operator curve and chi square test.

Results: Vaginal washing concentration of the four markers were significantly higher in the study group (p<0.01). The sensitivity, specificity, positive predictive value, negative predictive value and accuracy for each marker were: 98%, 94%, 94.23%, 97.92%, and 96% for ά fetoprotein; 100%, 92%, 92.59%, 100% and 96% for creatinine; 78%, 68%, 70.9%, 75.56% and 73% for prolactin and 84%, 68%, 72.41%, 80.95% & for β human chorionic gonadotropin respectively. Creatinine was found to be the most cost effective followed by ά fetoprotein.

Conclusion: ά fetoprotein is the most reliable marker for diagnosing premature rupture of membranes followed by creatinine, however creatinine is the most cost effective out of the four biomarkers.

INTRODUCTION
Rupture of fetal membranes before the onset of labour is termed as premature rupture of membranes (PROM) whereas, rupture of the membranes prior to 37 completed weeks is called preterm PROM (PPROM). PROM affects 3 to 18.5% of all pregnancies and in most cases happens without apparent cause.1

Early and accurate diagnosis of preterm PROM is important for implementing gestational age-specific obstetric interventions and thus improve perinatal outcome and minimize serious complications. Conversely, a false-positive diagnosis of preterm PROM may lead to unnecessary interventions, like hospitalization, antibiotics and corticosteroids administration, and even induction of labour. The diagnosis of PROM is easy when the rupture is obvious but difficult, indeed impossible when the rupture is slight. Traditionally the diagnosis of PROM has relied on patient’s history of fluid leakage, confirmed by the presence of pooling of amniotic fluid in vagina with speculum examination and alkaline vaginal pH detected by nitrazine paper or the presence of ferning pattern after microscopic examination of dried vaginal secretions. However these traditional methods cannot be applied to all patients with 100% accuracy. There are drawbacks of these classical methods like in case of long latent period, amniotic pooling cannot be seen on speculum examination but this does not exclude membrane rupture. Similarly reliability of nitrazine paper test is poor giving 9.4% false negative & 17.4% false positive result according to Friedman and Mc Elin’s study.2

Ultrasoundographic amniotic fluid index determination is helpful but not reliable, because oligohydramnios for any reason cannot be distinguished easily from decreased amniotic fluid as a result of PROM. Therefore false positive and false negative rates are high.

Cytological staining techniques to identify fetal lanugo, fat
globules and squamous cells are abandoned now because they take time, are technically difficult, require tools such as dye and microscopes that are not easily available and their false negative rate is high. Similarly, dye instillation which is considered as the gold standard is not the preferred method because it is invasive, takes time and dye may affect the fetus adversely.

Because of the limitations of available testing methods, investigators have sought alternative biochemical markers in amniotic fluid, such as prolactin3-5, alpha-fetoprotein3,6,7, 26, β-subunit of human chorionic gonadotropin3,7-12, fetal fibronectin6,13,14, diamine oxidase6,15,16, lactate17, creatinine7,18-21, urea17, and insulin growth factor binding protein-114,15,. Interest in assessment of these markers stems from their high concentrations in amniotic fluid compared with normal vaginal secretions. The tests are based on the identification in the cervicovaginal discharge of one or more of these biochemical markers that are present in the setting of PROM, but absent in women with intact membranes.

β hCG is a gycoprotein produced exclusively by syncytiotrophoblasts in the placenta.22 It is present in amniotic fluid, as well as maternal blood and urine at a concentration ranging from 2000-70,000mIU/ml.22 Also, it is secreted by the cervical glands and is present at a certain level in vaginal fluid.

Mammalian AFP is a single chain glycoprotein with a molecular mass ranging from 66-72 k D.23 It is initially synthesized by yolk sac, followed thereafter by fetal liver. After entering the fetal urine it is detected in the amniotic fluid.24 The concentration gradient between the fetal plasma AFP and maternal AFP is approximately 150 -200 fold.23 Thus a higher concentration of AFP in the amniotic fluid renders it to be a possible marker of PROM.

Prolactin is a single polypeptide chain i.e. produced by anterior hypophysis under the control of hypothalamus.25 During pregnancy prolactin is produced by the maternal and the fetal hypophysis and the decidua. Higher concentration of prolactin is found in amniotic fluid as prolactin actively participates in the control of amniotic fluid volume and osmolarity.24 It has been found that the prolactin level in amniotic fluid is approximately 5-10 times that in maternal circulation.25

Creatinine in amniotic fluid is mainly contributed by the fetal urine.26 The fetus starts excreting urine into the amniotic fluid at 8th to 11th week of gestation.26 The creatinine concentration ranges between 1.5 to 2.0 mg/dl after 36 weeks till term.26

Thus it can be concluded that the mentioned biochemical markers have a high amniotic fluid concentration and can be employed in diagnosing PROM in equivocal cases. The current study is therefore designed to evaluate the clinical practicability of using β hCG, AFP, prolactin and creatinine in diagnosing PROM and also to demonstrate the most reliable amongst these markers. As per the available literature our study is the first study comparing these four markers i.e β hCG, AFP, prolactin and creatinine in diagnosing PROM and the evaluating their cost effectiveness.

Materials and methods: It was a case- control study conducted in Safdarjung Hospital. A total of 100 pregnant women between 20 – 40 weeks of gestation were recruited and consented to this study. The patients in the study were taken from the Antenatal OPD and labour room and were grouped as: Group-A: 50 pregnant women with history of leaking per vaginum (study group). Group-B: 50 pregnant women with normal pregnancy (control group). All the patients had uncomplicated singleton pregnancies. There were no signs of pregnancy related diseases or any history of medical treatment. Vaginal washings were collected after a written informed consent from all the subjects. After confirming the absence of bloody discharge in the vagina, posterior fornix was irrigated with 3 ml of sterile saline using 5 ml syringe, with the same syringe the vaginal washing was aspirated from fornix and transported to the laboratory at room temperature. The samples were centrifuged and stored at -20C until assay. βhCG, prolactin and AFP were measured by using the Calbio Inc ELISA kits for the individual proteins. Creatinine was measured by using the Jaffe’s technique.

Statistical analysis: Results were tabulated and statistically analysed by using student’s t-test. The optimum cut off for the markers were determined by area under the receiver operative curve (a ROC). Using these cut off points the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficiency were calculated. The statistical significance was determined by using chi square test and statistical significance p<0.05 was considered. The cost effectiveness of each marker was analysed by taking into account the cost per test for each marker and their respective efficiency. The data was analysed using the SPSS statistical software version 12.0.
RESULTS

One hundred consenting women were evaluated, of whom 50 had history of leaking per vaginum while the remainder were normal pregnant women taken as control group. The demographic data for both groups are presented in table I.

There were no significant differences in gestational age and parity between the two groups. The mean values of the four markers were higher in the study group than in the control group:

- $\beta$hCG: 49.17 +/- 11.87 versus 29.48 +/- 9.41 m IU/ml;
- AFP: 57.08 +/- 7.25 versus 22.58 +/- 10.64 ng/ml;
- prolactin: 30.42 +/- 6.60 versus 19.06 +/- 5.55 mg/ml; and
- creatinine: 0.26 +/- 0.0663 versus 0.09 +/- 0.0414 mg/ml respectively (table II).

Receiver operator curve analysis indicated that the performance of all the four markers were different (fig.1). The area under the curve, cut off value and the sensitivity, specificity, PPV, NPV and efficiency are listed in table 3. Statistical information clearly indicated that of the four markers AFP had the highest diagnostic performance followed by creatinine.

The cost effectiveness of each marker was analysed considering the cost per test for each marker and their respective efficiency. Creatinine was the cheapest and also with the highest efficiency and hence was the most cost effective marker followed by AFP. The cost effectiveness of each marker is tabulated in table III. which shows that the marker with the lowest value of cost × (1-efficiency) is the most cost effective.

DISCUSSION

PROM is a significant obstetrical problem which can lead to infectious morbidity and imminent term or preterm labour. Failure of diagnosis can lead to complications such as chorioamnionitis and preterm birth and may result in serious repercussions, so it’s accurate diagnosis has great importance. Patient’s history alone is not always reliable and with traditional diagnostic techniques, some cases still remain unconfirmed and equivocal which is a perplexing situation for the clinician. Studies have therefore been conducted to test the diagnostic accuracy of alternative biochemical markers for PROM.

In our study we sought to establish the diagnostic accuracy of $\beta$hCG, AFP, prolactin and creatinine as markers of PROM. We found that the levels of the mentioned markers were significantly higher in the patients with PROM in comparison to those without PROM.

The clinical application of $\beta$hCG in diagnosing PROM has been supported by many investigators. Our study demonstrated $\beta$hCG cut off value of 37.06 mIU/ml and the sensitivity, specificity, PPV, NPV and efficiency of 84%, 68%, 72.41%, 80.95% & 76% respectively. This observation was similar to that demonstrated by Shahin et al.

AFP demonstrated a very high diagnostic performance in our study with all patients in our study group showing AFP values above the set cut off of 45.80ng/ml. The sensitivity, specificity, PPV, NPV and accuracy were found to be 98%, 94%, 94.23%, 97.92%, and 96% respectively. Similar findings were also reported by Ni et al and Gaucherand et al.

Prolactin was reported to have an average diagnostic performance with a cut off value of 23.56ng/ml. The sensitivity, specificity, PPV, NPV and accuracy were 78%, 68%, 70.9%, 75.56% and 73% respectively. Kariman et al, Shahin et al & Buyukbayrak et al also found prolactin as a useful marker for PROM.

Creatinine was reported to be a good indicator for PROM with 100% sensitivity, 92% specificity, 92.59% PPV, 100% NPV and 96% efficiency. This was almost similar to that shown by the other investigators like Zanjani et al, Sekhavat et al & Kafali et al. Creatinine in amniotic fluid is mainly contributed by the fetal urine and since its level remains constantly high in the amniotic fluid till term it exhibits a very high diagnostic performance.

The comparative evaluation of the 4 markers in our study demonstrates that out of all, AFP stands out to be the best followed by creatinine. $\beta$hCG was the least reliable amongst them and prolactin exhibited an average diagnostic performance. Highest sensitivity was reported for creatinine which was 100% followed by AFP which had 98% sensitivity. However the specificity was higher for AFP i.e. 94% as compared to creatinine which had 92% specificity. PPV was again the highest for AFP i.e. 94.23% followed by that of creatinine which had PPV of 92.59%. However NPV for creatinine was highest i.e. 100% followed by AFP which had NPV of 97.92%. So if no creatinine was found positive in the vaginal washings one need not doubt about any risk of PROM. Both AFP and creatinine had same efficiency of 96% and overall out of the two markers, AFP exhibited a slightly higher diagnostic performance followed by creatinine which was not far behind and can be regarded as a reliable indicator. Prolactin on the other hand had an average diagnostic performance. The least reliable of the four
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markers was βhCG.

The novel aspect of our study was to find the most cost effective amongst the four markers. This was analysed using the price of each marker per test and their respective efficiency. Creatinine was found to be the most cost effective with cost of Rs.100 per test and a high efficiency of 96%, followed by AFP which also had efficiency of 96% but its cost was higher i.e Rs.600 per test.

Currently, AFP finds application for multifarious purposes and is used as tumour marker, triple test screening for Down’s syndrome and neural tube defects, and is often available for daily assay in the tertiary care centres. As there is no need for extra equipment and reagent, introduction of this method into routine use is feasible and practical.

Creatinine on the other hand is a rapid, cheap and easily available test used for various purposes and since it has a high diagnostic performance it can be used particularly as an adjunctive test in equivocal cases of PROM when diagnosis is of great doubt. The best part about creatinine is that it is available even in the district health care centres and is the most cost effective thus making it an overall ideal marker for PROM. Moreover, during the study we found that the patients were compliant with method of sample collection and readily accepted this non invasive technique and therefore vaginal washing AFP and creatinine estimation can be employed as a reliable method for diagnosing PROM.

| Table 1 | Demographic data and the mean level of each marker in patients of study and control group. |
| PARAMETERS | STUDY GROUP | CONTROL GROUP | p value |
| Mean Gestational age at sampling in weeks | 35.53± 3.84 | 36.67± 3.84 | <0.05 |
| Parity | 0.88 ± 3.52 | 0.84± 4.60 | <0.05 |
| βhCG mIU/ml | 49.17± 11.87 | 29.48± 9.41 | <0.001 |
| AFP ng/ml | 57.08± 7.25 | 22.58± 10.64 | <0.001 |
| Prolactin ng/ml | 38.42± 6.60 | 19.06± 5.55 | <0.001 |
| Creatinine mg/ml | 0.26± 0.0663 | 0.09± 0.0414 | <0.001 |

<p>| Table 2 | Diagnostic performance of individual markers. |</p>
<table>
<thead>
<tr>
<th>Marker</th>
<th>Cutoff</th>
<th>sensitivity</th>
<th>specificity</th>
<th>Area under curve</th>
<th>PPV</th>
<th>NPV</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP mg/ml</td>
<td>45.80 ng/ml</td>
<td>94%</td>
<td>94%</td>
<td>0.931</td>
<td>94.2%</td>
<td>97.9%</td>
<td>96%</td>
</tr>
<tr>
<td>Creatinine mg/ml</td>
<td>0.1641 ng/ml</td>
<td>100%</td>
<td>92%</td>
<td>0.960</td>
<td>72.4%</td>
<td>100%</td>
<td>90%</td>
</tr>
<tr>
<td>Prolactin ng/ml</td>
<td>23.5 mg/ml</td>
<td>78%</td>
<td>68%</td>
<td>0.863</td>
<td>79.5%</td>
<td>75.5%</td>
<td>73%</td>
</tr>
<tr>
<td>βhCG mIU/ml</td>
<td>37.06 mIU/ml</td>
<td>84%</td>
<td>68%</td>
<td>0.855</td>
<td>80.95%</td>
<td>72.41%</td>
<td>70%</td>
</tr>
</tbody>
</table>

<p>| Table 3 | Cost effective analysis of each marker. |</p>
<table>
<thead>
<tr>
<th>Marker</th>
<th>Efficiency</th>
<th>1-Efficiency</th>
<th>Cost per test in Rupees</th>
<th>Cost - (1-Efficiency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP</td>
<td>96%</td>
<td>0.04</td>
<td>800</td>
<td>32</td>
</tr>
<tr>
<td>Creatinine</td>
<td>96%</td>
<td>0.04</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>Prolactin</td>
<td>73%</td>
<td>0.27</td>
<td>600</td>
<td>162</td>
</tr>
<tr>
<td>βhCG</td>
<td>76%</td>
<td>0.24</td>
<td>600</td>
<td>144</td>
</tr>
</tbody>
</table>

| Figure 1 | Composite ROC curve for βhCG, AFP, Prolactin & Creatinine. |

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