The value of the insulin-like growth factor binding protein-1 in the cervical–vaginal secretion detected by immunochromatographic dipstick test in the prediction of delivery in women with clinically unconfirmed preterm premature rupture of membranes

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Abstract

Objective: The aim of this study was to determine the value of detecting insulin-like growth factor binding protein-1 (IGFBP-1) in the cervical–vaginal secretion in the prediction of delivery in women with clinically unconfirmed preterm premature rupture of membranes (PPROM).

Material and methods: A total of 87 women, gestational age between 20 and 36 weeks were enrolled into this prospective study. Based on the clinical diagnosis, patients were grouped as clinically evident PPROM (n = 25), clinically suspected PPROM (n = 42) and women with intact fetal membranes (n = 20). Detection of IGFBP-1 in the cervical–vaginal secretions was done using a one-step immunochromatographic dipstick test in all women. The outcome measures were gestational age at delivery, neonatal birth weight and duration of the interval between the test and delivery between women with positive and negative test results.

Results: The test was positive in all 25 women (100% sensitivity) with clinically evident PPROM and all delivered prematurely, and negative in 19 out of the 20 (95% specificity) women with intact fetal membranes. Among 36 women with clinically suspected PPROM, 13 (36%) tested positive and 23 (63%) tested negative for IGFBP-1. In this group, the mean gestational age and birth weight at the time of delivery were significantly lower in patients with positive test (31.38 ± 2.6 weeks versus 38.61 ± 0.99 weeks and 1761 ± 527 g versus 3500 ± 355 g, P < 0.05 for both). Eleven (85%) of the 13 women with positive test, delivered within 2 weeks after the performance of the test whereas all the women with negative test results delivered after 2 weeks (P = 0.001). The test had 100, 92, 84 and 100% sensitivity, specificity, positive predictive value and negative predictive value, respectively, for the outcome measure of test–delivery interval.

Conclusion: The screening test for IGFBP-1 in the cervical–vaginal secretions is a useful adjunct in the prediction of delivery in women with clinically unconfirmed PPROM.

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1. Introduction

Premature rupture of membranes complicates 3–19% of all pregnancies and is responsible for approximately 30% of all preterm deliveries [1]. Pregnanies affected by premature rupture of membranes are associated with increased risk for umbilical cord prolapse, abruptio placentae, chorioamnionitis, and postpartum endometritis. The fetus is also at risk of developing fetal compression syndrome (Potter syndrome) and pulmonary hypoplasia [2]. Preterm premature rupture of amniotic membrane is of particular importance because of its association with a latency period from membrane rupture to delivery. Mostly, the diagnosis is established based on the history and clinical findings. However, a reliable diagnosis is

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clinically not possible in about 10% of the cases [3]. Although various tests have been used in the diagnosis of premature rupture of membrane, most of these tests lack the necessary sensitivity and specificity, especially in cases in which the diagnosis of ruptured amniotic membranes is clinically doubtful [4]. To optimize perinatal outcome by premature rupture of membranes, a rapid test with a high accuracy rate is required.

Insulin-like growth factor I and II are ubiquitous mitogens that affect cell growth and metabolism [5]. In biological fluids they are normally bound to specific protein, designated 1–6 [6,7]. Insulin-like growth factor binding proteins modulate the interaction between insulin-like growth factor and their receptors, and they have different functions. IGFBP-1 constitutes a subgroup of the insulin-like growth factor binding protein systems, which reflects the acute changes of insulin-like growth factor in the plasma and regulates cellular growth and metabolism [6,7]. Insulin-like growth factor binding protein is secreted from human liver, decidual cells and placenta. Its concentration in the amniotic fluid is considerably higher than the concentration in other body fluids. It is the major insulin-like growth factor binding protein in the amniotic fluid that gradually increases in the second trimester and remains higher throughout pregnancy in comparison to its plasma levels [8–10]. Detection of IGFBP-1 in the cervical–vaginal secretions has been shown to be a reliable method in the diagnosis of ruptured amniotic membrane in cases in which the clinical diagnosis is uncertain [11–14].

In a previous study we determined the value of cervical phosphorylated IGFBP-1 by a similar immunochromatographic dipstick test in the prediction of preterm labor in women presenting with threatened preterm labor. The IGFBP-1 test was found negative in all the patients in the control group (n = 20), and all these patients delivered after 37 weeks, while the test was positive in 15 of the 45 (33.3%) patients in the preterm delivery group. The sensitivity, specificity, positive predictive value, and negative predictive value for the IGFBP-1 test were found 78, 87, 73 and 90%, respectively, for the preterm delivery. The high negative predictive value of the test may be of value in the avoidance of unnecessary medical interventions for the preterm labor [15].

In the present study we determined the accuracy of detecting cervical–vaginal secretion in the prediction of delivery in women with clinically unconfirmed preterm premature rupture of membranes (PPROM).

2. Material and methods

Eighty-seven consecutive pregnant women, gestational age between 20 and 36 weeks admitted to Department of Obstetrics and Gynecology, Ege University Faculty of Medicine between February 2001 and November 2002 were enrolled into this prospective study. Verbal informed consent was obtained from all patients after the detailed explanation of the diagnostic procedure.

All patients were evaluated for ruptured amniotic membranes by history taking and vaginal speculum examination for the evidence of fluid leakage. The attending clinician made the clinical diagnosis of ruptured amniotic membranes (clinically evident premature rupture of membranes) or no ruptured amniotic membranes (women with intact fetal membranes). Women presented with ongoing vaginal fluid leakage and/or ruptured amniotic membranes at first vaginal examination were considered clinically evident PPROM. Those without any history of fluid leakage as supported by clinical evaluation were regarded as pregnant women with intact fetal membranes. The diagnosis of suspected rupture of membrane was made in those patients with a history of fluid leakage but could not be excluded or confirmed clinically (with no pooling of amniotic fluid in the posterior fornix). Based on the clinical diagnosis, patients were grouped as clinically evident PPROM (n = 25), clinically suspected PPROM (n = 42) and women with intact fetal membranes (n = 20). Six of the 42 women with suspected ruptured amniotic membranes were eliminated from the study. Four of these women tested negative for IGFBP-1 were lost on follow-up, and two women with positive test results refused hospital admission.

Patients presenting with a history of vaginal bleeding, history of amniotic fluid leakage for more than 7 days, twin gestations, cervical dilatation of more than 2 cm or effacement more than 80% were not taken into the study.

Detection of IGFBP-1 in the cervical or vaginal secretions was done using a one-step, dipstick test (Actim PROM test®, Medi Diagnostics, Kauniainen, Finland) in all groups. The test was performed by placing the lower end of the dipstick into the external cervical orifice and then the posterior fornix, after a sterile speculum was inserted into the vagina. The dipstick was left in place for approximately 10–15 s, so that secretions were absorbed. Then the dipstick was placed immediately into the extraction solution containing test tube where it was left for approximately 15–20 s to allow the liquid front to enter the result area. The results were interpreted by waiting for 5 min, while holding the dipstick in a horizontal position.

The Actim PROM test is an immunochromatographic qualitative test for detection of amniotic fluid leakage which involves two monoclonal antibodies to human IGFBP-1. One antibody is bound to blue latex particles (the detecting label). The other is immobilized on a carrier membrane to catch labeled particles and indicate a positive result. When the dip area of the dipstick is placed in an extracted sample, the dipstick absorbs liquid, which start to flow up the dipstick. If the sample contains insulin-like growth factor binding protein-1 it binds to the antibody attached to the latex particles. The particles are carried by the liquid flow and, if insulin-like growth factor binding protein-1 is bound
to them, they bind to catching antibody. A blue line appears in the result area if the concentration of insulin-like growth factor binding protein-1 in the sample exceeds the cut-off value for the test (positive test). A second line was used to confirm the performance of the test. The insulin-like growth factor binding protein-1 concentration higher than 50 mcg/L in the extracted sample gave a strong positive, whereas, concentration of 25 mcg/L gave a weak positive result.

The results were said to be positive, negative or invalid, when two, one, or no blue line(s) appear(s), respectively, on the dip area of the dipstick. The extraction solution is a phosphate-buffered solution which contains bovine serum albumin, protease inhibitors and preservatives.

Data analysis was compared only in patients with suspected rupture of membranes which were taken as study group. Mann–Whitney test was used to compare the data. Data presented as percentages were compared with chi-square test. \( P \) value lower than 0.05 was accepted as significant.

### 3. Results

The test was positive in all 25 (100% sensitivity) women with clinically evident premature rupture of amniotic membranes and negative in 19 out of the 20 (95% specificity) pregnant women with intact fetal membranes. One woman with intact fetal membrane had a weak positive result which was regarded as a positive result. All women with clinically evident premature rupture of amniotic membranes delivered prematurely.

In the remaining 36 women with clinically suspected rupture of membranes, 13 (36%) tested positive and 23 (63%) tested negative for IGFBP-1. There were no significant differences when age and parity were compared between these two groups \( (P = 0.05) \). When gestational age at time of delivery and neonatal birth weight in women with positive results were compared to those with negative results, significant difference was observed \( (P < 0.05) \). Comparison between the two groups is represented in Table 1. The mean gestational age at the time of delivery was 31.3 ± 2.6 and 38.6 ± 0.9 weeks in patients with positive and negative test results, respectively. The mean neonatal birth weight was 1761 ± 527 and 3500 ± 355 g in patients with positive and negative test results, respectively. Eleven (85%) of the 13 women with positive test, delivered within 2 weeks after the performance of the test whereas all the women with negative test results delivered after 2 weeks \( (P = 0.001) \). When the diagnosis of premature rupture of membrane was in doubt clinically, detection of IGFBP-1 in the cervical–vaginal secretions by a rapid dipstick was found to have 100, 92, 84 and 100% sensitivity, specificity, positive predictive value and negative predictive value, respectively, for the outcome measure of test–delivery interval.

### 4. Discussion

Premature rupture of the membrane remains a significant obstetric problem. Preterm premature rupture of the membrane is an important cause of perinatal morbidity and mortality, and is responsible for about one-third of all preterm births [1]. In order to reduce perinatal morbidity and mortality associated with preterm premature rupture of membrane, timely evaluation and management is of utmost importance.

Although almost 90% of the cases is confirmed clinically, the accuracy of most tests used to confirm rupture of membranes when the clinical diagnosis is doubtful, are limited by the presence of various confounding factors [4,15]. Although amniotic fluid crystallization test (Fern test) is unaffected by meconium, changes in vaginal pH, and blood–amniotic fluid ratios up to 1:5, collection of cervical mucus may produce false-positive results, while maternal blood and vaginal discharge may lead to false-negative results [16,17]. A rise in vaginal pH caused by blood, semen contamination, alkaline antiseptics, or bacterial vaginosis may result in false-positive, whereas the presence of scant amount of amniotic fluid may result in false-negative nitrazine test [4]. The usage of ultrasonography alone cannot diagnose or exclude membrane rupture with certainty, although oligohydramnios without evident fetal urinary tract malformations or fetal growth restriction may be suggestive of membrane rupture [18]. An unequivocal confirmation of membrane rupture by ultrasound-guided amnioinfusion of indigo carmine dye followed by observation for passage of blue fluid per vaginum is limited by the invasiveness of the test [18]. Since a positive test may reflect disruption of decidual rather than amniotic membrane rupture, cervical–vaginal screening for fetal fibronectin is not recommended for routine practice in the diagnosis of preterm premature rupture of membranes [18].

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The data in patients with clinically suspected rupture of membranes</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IGFBP-1 screening positive ( (n = 13) )</td>
</tr>
<tr>
<td>Age</td>
<td>26.4 ± 4.5</td>
</tr>
<tr>
<td>Week of performance of the screening test</td>
<td>30.8 ± 2.6</td>
</tr>
<tr>
<td>Parity</td>
<td>0.8 ± 0.9</td>
</tr>
<tr>
<td>Delivery week</td>
<td>31.3 ± 2.6</td>
</tr>
<tr>
<td>Birth weight</td>
<td>1741 ± 527</td>
</tr>
</tbody>
</table>

IGFBP: insulin-like growth factor binding protein.
In the present study we evaluated the value of detecting IGFBP-1 in the cervical–vaginal secretion in the diagnosis of premature rupture of membrane in clinically doubtful cases. The Actim PROM test is a rapid bedside test, developed to detect the presence of IGFBP-1 in the cervical–vaginal secretions. IGFBP-1 is the major insulin–like growth factor binding protein in the amniotic fluid that gradually increases in the second trimester and remain higher throughout pregnancy in comparison to its plasma levels [8–10]. Its concentration in amniotic fluid is 100–1000 times higher than the concentration in other bodily fluids [10]. Its phosphorylated isoform is mainly secreted in the decidual cells and human liver, whereas the nonphosphorylated isoform is mainly found in the amniotic fluid [9,10].

In our study we were able to demonstrate that patients with suspected diagnosis of premature rupture of membrane, suggested by the report of wetness or leakage of fluid, who have positive IGFBP-1 detecting test were more likely to deliver within 2 weeks after the performance of the test. The test was found to have 100% sensitivity, 92% specificity, 84% positive predictive value and 100% negative predictive value in the identification of women with equivocal diagnosis of premature rupture of membranes for the outcome measure of test–delivery interval.

Studies have shown that detection of IGFBP-1 in the cervical–vaginal secretion, have the sensitivity of 100% and specificity of 95% in the confirmation of rupture of membrane [11–14]. Rutanen et al. [13] reported 100% sensitivity in cases of unequivocal rupture of membrane. In their study they found that women with equivocal rupture of membrane and positive test for IGFBP-1 had a 6.9-fold increase in relative risk of preterm delivery, compared to women who had negative test result at the time of evaluation. They also stated that, the IGFBP-1 test was an independent predictor of preterm delivery \( (P = 0.0001) \). The presence of confounding factors such as vaginal infection, discharge, medications, urine or seminal fluids were found to have no effect on the performance of the test [13]. However, in the presence of heavy vaginal bleeding, the amount of IGFBP-1 in the blood may be so high to give a positive result [19], whereas cessation of amniotic fluid leakage for more than 12 h before specimen collection, may give a false negative result [11]. In our study, patients presented with history of vaginal bleeding were excluded from the study, as were patients with premature uterine contractions to avoid false positive results from plasma and decidual IGFBP-1. Patients with history of membrane rupture for more than 7 days were excluded from the study since their pregnancies were terminated following the admission.

In the present study, the accuracy of the test in the group with suspected PROM was assessed by comparing the end-points of gestational age at delivery, neonatal birth weight, and duration of the interval between the test and delivery among the women with positive and negative test results. These end-points are indirect evidence of PROM. Although we use ultrasound routinely for evaluation of the amniotic fluid, in this study, we did not take amniotic fluid volume into consideration as it is less reliable especially in women with clinically unconfirmed preterm premature rupture of membranes (PPROM). One of the limitations of the study is that there is no ‘gold-standard’ that truly reflects the presence of fluid in the vagina such as the intra-amniotic injection of indigo carmine. And the other limitation of the study is that we could not compare the test to any traditional methodology used for diagnosis of PPROM-like vaginal PH Fern test (amniotic fluid arborization). So, it cannot be really known if this test is more accurate and sensitive.

In conclusion, detection of IGFBP-1 in the cervical–vaginal secretions by a rapid test is to be of value and clinically useful in the identification of women with suspected rupture of membranes in whom the clinical diagnosis was not established with certainty.

References


