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Evaluation of a rapid strip test for insulin-like growth factor binding protein-1 in the diagnosis of ruptured fetal membranes

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Abstract

We evaluated the clinical usefulness of a new bedside test (PROM TEST) for insulin-like growth factor binding protein-1 (IGFBP-1) in the detection of ruptured fetal membranes (ROM). Cervicovaginal secretion was sampled between 15 and 37 weeks of gestation from asymptomatic women with apparently intact membranes and from women with clinically confirmed ROM, as well as from symptomatic women with suspected ROM based on history. IGFBP-1 in samples was detected with a dipstick based on immunochromatography. The test result was positive in 100% of cases with unequivocal ROM and in 5.3% of cases with apparently intact membranes. Furthermore, the PROM TEST was positive in 64 of 181 patients evaluated for suspected ROM based on history, but in whom the diagnosis could not be clinically confirmed at the initial evaluation. Fifty of the 64 women (78.1%) were delivered prematurely (< 37 weeks). Five of the 117 PROM-negative patients had elective cesarean section for reasons unrelated to ROM before 37 weeks and 10 of the remaining 112 patients (8.9%) had preterm delivery. Women with equivocal ROM and a positive test result had a 6.9-fold increased relative risk (95% confidence interval 4.2–11.4) of preterm delivery compared with women who had a negative result at

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the time of evaluation. Multiple logistic regression including PROM TEST result, contractions, vaginal bleeding and cervical changes indicated that a positive PROM TEST result was an independent predictor of preterm delivery ($P = 0.0001$). In summary, a positive PROM TEST result identifies ROM with high sensitivity and a negative result effectively excludes those with intact membranes. In patients with suspected but clinically unconfirmed ROM, the positive test result is associated with increased risk of preterm delivery, suggesting that microruptures of fetal membranes can also be detected by the PROM TEST.

Keywords: PROM; Amniotic fluid; Insulin-like growth factor binding protein-1 (IGFBP-1); Preterm delivery

1. Introduction

Premature rupture of fetal membranes (PROM) occurs in 4.5–7.6% of pregnancies [1,2]. When occurring before 37 weeks of gestation, PROM is associated with increased incidence of amnionitis and prematurity and by increased fetal and maternal morbidity and mortality. The patient's history and physical examination alone are often inadequate to confirm the diagnosis of PROM and ultrasound examination provides help to the diagnosis only when a large amount of fluid has escaped. The leakage of amniotic fluid may be intermittent and there may be no fluid present in the vagina at the time of examination, or the vaginal fluid may be contaminated with urine, cervical secretion, blood or semen. To date no single test has been developed which is found to be wholly accurate in detecting fetal membrane rupture (ROM) in such clinical conditions. We have previously reported that insulin-like growth factor binding protein-1 (IGFBP-1) is an ideal marker for amniotic fluid and its detection in cervical/vaginal fluid was found to be valuable for the diagnosis of ROM [3]. Recently, Lockwood et al. [4] reported similar results using the same immunoenzymometric assay that we used for quantitation of IGFBP-1. IGFBP-1, previously known as placental protein 12, is produced by decidual cells [5]. It is a major protein in amniotic fluid, where the average concentrations are 100- to 1000-fold higher than those in serum throughout gestation [3,6]. The protein is barely detectable in urine, cervical mucus and seminal fluid which all may mimic amniotic fluid in the vagina [3].

Here we evaluate the clinical utility of a rapid strip test (PROM TEST; Medix Biochemica, Kauniainen, Finland) which detects IGFBP-1 in cervicovaginal secretion when the level is above the 95th percentile of serum levels in the detection of fetal membrane rupture.

2. Patients and methods

2.1. Patients

A multicenter study was conducted at the Departments of Obstetrics and Gynecology of Helsinki, Oulu and Tampere University Hospitals and Helsinki City Maternity Hospital, Finland. A sterile dacron swab sample was obtained at the speculum examination from the external os of the cervix of 75 women without clinical evidence of ROM and with no signs or symptoms of premature labor at routine prenatal visit between 15 and 37 weeks of gestation. Another set of cervical samples was obtained from 55 women up to 144 h after clinically confirmed ROM. In addition, cervical samples were obtained between 18 and 37 weeks of gestation from 181 women presenting at the maternity clinic unscheduled, with a history of leakage of fluid from the vagina but in whom the diagnosis of ROM could not be clinically confirmed (negative pooling) at the time of initial evaluation. Gestational age was determined on the basis of the last menstrual period and confirmed by early second trimester ultrasound evaluation. The history and findings of clinical examination, including uterine contractions, cervical dilatation and vaginal bleeding were carefully documented at the time of sampling. All patients had given informed consent and the samples were obtained with approval of the Ethical Committee.

2.2. PROM TEST

The strip test for detection of IGFBP-1 (PROM TEST) in cervical/vaginal secretion was manufactured by Medix Biochemica, Kauniainen, Finland. The PROM TEST is based on the use of two monoclonal antibodies to human IGFBP-1 and immunochromatography [7,8]. In the dipstick, one of the antibodies is immobilized on blue latex particles (the detecting label), the other is bound to the membrane and acts as the specific IGFBP-1 catching line. When the absorbing end of the dipstick is placed in the extracted specimen, IGFBP-1, if present, binds to antibody labelled blue particles which move until bound by the second antibody in the catching line. The dipstick also contains a line with immobilized anti-mouse immunoglobulins which always catches labelled blue latex particles to form a blue line, irrespective of the presence or absence of antigen in the sample. Thus, one blue line on the dipstick confirms that the test has been performed correctly and two blue lines indicate that the sample contains IGFBP-1 above the adjusted detection limit. A sterile dacron swab, a test tube containing 0.5 ml of extraction buffer (phosphate-

buffered solution containing bovine serum albumin, detergent, protease inhibitors and preservative) and a dipstick are included in the test kit. The detection limit is adjusted to discriminate between serum IGFBP-1 and amniotic fluid IGFBP-1 [3]. Because the dacron swab absorbs about 150 μl of fluid when saturated, the average dilution of cervicovaginal sample in the buffer is about 1 to 5. Extraction efficiency of IGFBP-1 from the swab varies from 20 to 60%, depending on the IGFBP-1 content of the sample. The detection limit of the PROM TEST has been adjusted such that an IGFBP-1 concentration of 25 μg/l in the extracted sample gives a weak positive line and concentrations above 50–100 μg/l give a strong positive result. Considering both the absorption capacity of the swab and the extraction efficiency of the protein from it, IGFBP-1 concentrations above 400 μg/l (above the 95th percentile of serum levels) [3,6] in the unextracted sample will be detected by the dipstick. IGFBP-1 concentrations up to 200 000 μg/l show no high dose hook effect in the test. Urine and seminal plasma or various vaginally administered drugs do not interfere with the test [3]. Color due to the hemolysis of red cells does not interfere with the performance of the test, but in cases with heavy bleeding of decidual origin the content of IGFBP-1 in the serum may, in theory, be high enough to cause a positive reaction. Therefore, the positive result should be interpreted with caution in cases with heavy vaginal bleeding.

2.3. Sampling and performance of the test

All samples were collected using the PROM TEST kit. After leaving in the cervix for about 15 s the dacron swab was placed in a test tube containing 0.5 ml of extraction buffer and rinsed in buffer for approximately 15 s. The swab was then withdrawn and the dipstick was placed into the tube and allowed to absorb the extract until the liquid front was visible in the reaction area (about 1 min). A positive result appears in 2–5 min as two blue lines on the dipstick. A negative result (one blue line) can be confirmed in 5 min.

2.4. Immunoenzymometric assay (IEMA) for IGFBP-1

To control how the adjusted detection limit of the PROM TEST works, a total of 281 buffer tubes were stored after the strip test at −20°C until the IGFBP-1 concentration in the remaining buffer was measured by a sensitive immunoenzymometric assay (IGFBP-1 IEMA TEST, Medix Biochemica, Kauniainen, Finland) as described previously [3]. All samples were measured in duplicate. The detection limit of the assay is 0.4 μg/l. The intra- and interassay coefficients of variation were 3.4% and 7.4%, respectively.

2.5. Statistical analysis

The data were analyzed using BMDP (Los Angeles, CA) and SAS (SAS Institute Inc., Cary, NC). Student's unpaired t-test was used for continuous variables (age, weeks of gestation) and differences in the distribution of discrete variables were analyzed using Fisher's exact test. Multiple logistic regression was used to analyze whether a positive PROM TEST result in patients with equivocal ROM was independently associated with preterm delivery when contractions, bleeding and cervical dilatation were included as independent risk factors in the model. $P < 0.05$ was considered statistically significant.

3. Results

All samples ($n = 55$) taken 1–144 h after clinically confirmed ROM showed a positive result and 71 of 75 samples (94.7%) taken from asymptomatic women between 15 and 36 weeks of gestation were negative according to the PROM TEST. Among this set of samples, the positive PROM TEST result had a sensitivity of 100% (95% confidence interval 93.5–100%) and a specificity of 94.7% (95% confidence interval 86.9–98.5%) in detection of fetal membrane rupture. In the absence of any non-invasive test that could with 100% certainty separate microrupture from non-ruptured fetal membranes in uncertain cases, the PROM TEST results of patients with suspected, but equivocal, PROM were evaluated in relation to the interval between sampling and delivery.

Among the 181 patients evaluated for suspected, but at initial examination equivocal, ROM between 18 and 36 weeks of gestation the PROM TEST was positive in 64 cases and negative in 117 cases. Table 1 shows the demographic and obstetric characteristics of the patients. There were no significant differences in maternal age, parity and estimated gestational age at the time of evaluation between the PROM TEST-positive and negative groups. The proportion of patients with vaginal bleeding and cervical changes was significantly greater in the PROM TEST-positive group than in the PROM TEST-negative group. In contrast, women with a negative PROM TEST result had more frequent contractions than the PROM TEST-positive patients. Table 2 shows the clinical characteristics of the patients according to PROM TEST results. Among the 64 patients with a positive PROM TEST, the median time between sampling and delivery was 6 days (range 0–145). Fifty patients (78.1%) were delivered before 37 weeks of gestation, 42 (65.6%) within 2 weeks after sampling. Thirteen patients (26%) were delivered by caesarean section and four were induced due to signs of chorioamnionitis. Five of 117 patients with a

Table 1
Demographic and obstetric characteristics of 181 women with suspected but equivocal ROM grouped according to PROM TEST results

Variable	PROM TEST-positive ($n = 64$)	PROM TEST-negative ($n = 117$)	Significance
Maternal age (years)	30.7 (S.D. 4.6)	29.6 (S.D. 5.3)	NS[a]
Parity > 0	34/64 (53.1%)	68/117 (58,1%)	NS[b]
Cervical dilatation ⩾ 2 cm	7/64 (10.9%)	5/117(4.3%)	NS[b]
Vaginal bleeding	16/64(25.0%)	16/117 (13.7%)	NS[b]
Contractions	8/64 (12.5%)	32/117 (27.4%)	$P = 0.034$[b]
Chorioamnionitis (clinical) or postpartum endometritis	10/64 (15.6%)	1/117 (0.9%)	$P = 0.0002$[b]

Age are means and (S.D.). NS not significant.
[a]Student's *t*-test,
[b]Fisher's exact test.

Table 2
Clinical characteristics of 181 women with equivocal ROM at initial examination grouped according to PROM TEST results

Variable	PROM TEST-positive ($n = 64$)	PROM TEST-negative ($n = 117$)	Significance
Gestational age at time of suspected rupture of membranes (weeks)	29.1 (S.D. 5.8)	30.3 (S.D. 4.4)	NS[a]
Gestational age at delivery (weeks)	32.6 (S.D. 5.6)	38.1 (S.D. 2.1)	$P < 0.0001$[a]
Preterm delivery	50/64 (78.1%)	10/112 (8.9%)	$P < 0.0001$[b]
Time from sample to delivery ⩽2 weeks	42/64 (65.6%)	7/112 (6.3%)	$P < 0.0001$[b]

[a]Student's *t*-test;
[b]Fisher's exact test; preterm delivery ⩽ 37 weeks.

negative PROM TEST had elective cesarean section before 37 weeks for reasons unrelated to PROM. Among the other 112 patients, 102 (91.1%) delivered at term and 10 (8.9%) were delivered < 37 weeks, seven of those (6.3%) within 2 weeks alter sampling. One of the 10 preterm deliveries (10%) was cesarean section for chorioamnionitis 11 days after initial

evaluation. The median time from sampling to delivery in the PROM TEST-negative group was 54 days (range 2–158). Women with suspected ROM and a positive test result had a 6.9-fold increased relative risk (odds ratio 36.3; 95% confidence interval 4.2–11.4) of preterm delivery compared with women who had a negative result at the time of evaluation. After contractions, vaginal bleeding and cervical dilatation were included in a multiple logistic regression model, a positive PROM TEST result was shown to be a highly significant independent predictor of preterm delivery ($P = 0.0001$, odds ratio 44.3, 95% confidence interval 16.2–121.1).

After the dipstick test, IGFBP-1 concentrations were measured by IEMA in 281 extraction buffers. In 159 of 160 PROM TEST-negative samples, the IGFBP-1 concentration in extraction buffer was < 25 μg/l (mean: undetectable; 95% confidence interval undetectable to 16.8 μg/l). In 121 PROM TEST-positive samples the IGFBP-1 concentration ranged from 4.6 μg/l to 110000 μg/l (median: 110 μg/l; 95% confidence interval 9.2–31.000 μg/l) and in 85% it was >25 μg/l.

4. Discussion

If the diagnosis of fetal membrane rupture remains uncertain by physical examination, the ideal method for demonstrating the presence of amniotic fluid in the vagina should be rapid and available to the examining clinician 24 h a day. It should accurately discriminate between amniotic fluid and other fluids which may contaminate amniotic fluid in the vagina. Here we demonstrate that the PROM TEST based on the detection of IGFBP-1 in cervical secretion possesses the advantages listed above. The test can be performed even though no fluid is visible in the vagina. No special equipment or laboratory staff is required and the test result can be obtained within a few minutes at the beside.

The marked gradient between blood and amniotic fluid IGFBP-1 concentrations makes IGFBP-1 a potential marker for detection of ROM and makes the development of the dipstick test possible. Bloody show may contaminate amniotic fluid in the vagina but does not appear to interfere with the test. Samples contaminated with blood were found among PROM TEST-positive and negative samples. The detection limit of the PROM TEST was set so that IGFBP-1 concentrations below 400 μg/l in cervical secretion, i.e. below the 95th percentile of serum IGFBP-1 levels in pregnant women, should remain negative. However, in cases with heavy bleeding, the test result should be interpreted with caution as blood straight from the placental bed may contain higher amounts of IGFBP-1 than blood from the cervical blood vessels.

The most commonly used tests in the diagnosis of ROM, the Nitrazine test and the arborization test [1], are not in routine use in Finland. Because no test is available that could with 100% certainty detect ruptured fetal membranes, especially in the case of microrupture, the sensitivity and specificity values for this type of test are always difficult to calculate. However, the 100% positive result in clinically confirmed ROM and the 94.7% negative result in cases with apparently intact membranes, as obtained with the PROM TEST, are very acceptable for any clinical test.

It is well established that patients with ROM have an increased risk of preterm delivery. In a study by Taylor and Garite [9], the median length of time from PROM to delivery was 6 days (range 1–87) in 53 women having PROM at 16–25 weeks. In the present study, the median time from sampling to delivery was also 6 days in patients who were evaluated for suspected ROM and who had a positive PROM TEST result. Most of these patients were admitted which, if it had any effect, would increase the time period from sampling to delivery. Since this was the case, it is likely that in this group of patients the positive PROM TEST result reflected leakage or transudation of amniotic fluid into the vagina. This leakage may have been transient, as shown in some patients in whom the test result was negative a few weeks after the initial test (data not shown) and who delivered at term. On the other hand, most of the patients with a negative PROM TEST but preterm delivery had more than 2 weeks between sampling and delivery. Histology has revealed areas of weakness in the membranes over the internal os and in the area directly across from the placental site [10], suggesting that whatever triggers PROM, membrane rupture may occur at or outside the internal os. The incidence of small membrane ruptures above the cervix is not known. Such patients may not be at the same risk for complications as patients with rupture in the lower pole of the amniotic sack and heavy PROM. However, even occult membrane rupture or simply transudation of amniotic fluid through the membranes into the vagina may indicate an increased risk of preterm delivery or intrauterine infection, warranting careful follow-up during the rest of pregnancy.

The IGFBP-1 concentrations in PROM TEST-negative sample buffers were in good agreement with the adjusted detection limit of 25 μg/l in an extracted sample. The effects of freezing and thawing and exposure to room temperature for a longer period may explain why slightly more sample buffers showing a positive test result immediately after sampling had an IGFBP-1 concentration < 25 μg/l as measured later from the frozen sample by IEMA. The same reasons may account for the lower cut-off for cervical fluid IGFBP-1 in cases with ruptured membranes reported by Lockwood et al. [4]. In our previous study we showed that

proteases may affect the IGFBP-1 concentration in the vagina and lower the IGFBP-1 concentration in vaginal secretion if a long time has elapsed since membrane rupture [3]. Lockwood et al. [4] could not confirm the protease effect and, to date, no proteases specific to IGFBP-1 have been detected.

Other proteins present in the amniotic fluid, including prolactin and alfa-fetoprotein have also been suggested as markers of amniotic fluid [11–13]. A low concentration gradient between amniotic fluid and serum especially during the third trimester limits the value of these proteins as markers of amniotic fluid in bloody vaginal secretions. Fetal fibronectin is present in amniotic fluid at all gestational ages, but also in this case the amniotic fluid/serum ratio is much lower than that for IGFBP-1 [14,15]. Another great advantage of the IGFBP-1 measurement over fibronectin is that IGFBP-1 tests are not affected by seminal plasma and can, therefore, be used even after recent sexual intercourse which frequently precedes the suspicion of ROM.

False positives for the fetal fibronectin test (ROM-check) were attributed to leakage of amniotic fluid through stretched fetal membranes [14]. Fetal fibronectin has been localized to the chorionic layer of fetal membranes and IGFBP-1 is produced by decidual cells adjacent to the chorionic membrane [3]. Due to the close proximity of the cells containing fetal fibronectin and IGFBP-1, it is possible that both proteins are leaking into the vagina on occasions when the cervix dilates and the chorion is detaching from the decidua in the lower uterine segment. In this study, cervical dilatation had no clear-cut effect on the test result and, unexpectedly, the proportion of patients with contractions was greater in the PROM TEST-negative group. IGFBP-1 secretion from ectopic decidual cells [16,17], which are occasionally present in the cervix, may provide one explanation for transiently positive PROM TEST results in patients in whom no other signs of ROM could be demonstrated and whose pregnancies continued without complications.

The PROM TEST, like other tests used for detection of amniotic fluid in the vagina, is affected by sample collection. Obviously the amount of fluid absorbed into a dacron swab depends on the time the swab has been held in the cervix. Another variable is the extraction of the protein from the swab into the buffer and reading of the result can be erroneous. By following instructions closely the effects of these variables can be minimized.

In summary, our data show that the PROM TEST based on the detection of IGFBP-1 in cervical secretion provides an additional diagnostic tool for evaluating patients who present with suspected but equivocal ROM. The test detects amniotic fluid in the vagina with high sensitivity.

In cases with equivocal PROM, a positive test result is associated with preterm delivery, suggesting the presence of microrupture. In contrast, a negative test result excludes patients with ROM and risk of preterm delivery with high accuracy.

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