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Measurement of insulin-like growth factor binding protein-1 in cervical/vaginal secretions: comparison with the ROM-check Membrane Immunoassay in the diagnosis of ruptured fetal membranes

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Summary

Insulin-like growth factor binding protein-1 (IGFBP-1) is a major protein in amniotic fluid. In this study, we evaluated the diagnostic potential of IGFBP-1 measurement in cervical/vaginal secretions as an indicator of ruptured fetal membranes. Data were also compared with those obtained by the ROM-check Membrane Immunoassay (Adeza Biochemical, Sunnyvale, California) that is based on the detecton of fetal fibronectin in vaginal fluid. In women with intact membranes and not in labor, the concentration of IGFBP-1 in specimens obtained from the cervix and immersed in 0.5 ml of assay buffer ranged from <0.5 to 90 μg/l, whereas in specimens obtained less than 8 h after spontaneous or artificial rupture of membranes it varied between 175 and 20,000 μg/l, the median being 1,900 μg/l. The values greater than 100 μg/l were interpreted as containing amniotic fluid. The IGFBP-1 measurement and the ROM-check Membrane Immunoassay were carried out parallel in the vaginal swab specimens obtained from 54 pregnant women from 1 h to 1 week after the rupture. Twenty-four women had ROM confirmed from 1 h to 1 week earlier. In this group of patients, IGFBP-1 concentration >100 μg/l had a sensitivity of 75% and a specificity of 97% in diagnosis of ROM. The corresponding numbers for a positive ROM-check were 92% and 80%. The positive predictive value

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was 95% for the IGFBP-1 measurement compared to 79% for the ROM-check test. These data show that IGFBP-1 is an ideal marker for amniotic fluid and rapid tests for its measurement in vaginal fluid might provide a reliable tool for detection of ruptured fetal membranes, if not more than 12 h have elapsed since the leakage of amniotic fluid into the vagina.

Introduction

Premature rupture of fetal membranes (PROM) occurs between 4.5% and 14% of pregnancies [1,2]. PROM is associated with a high incidence of amnionitis and prematurity and by increased fetal and maternal morbidity and mortality. The primary problem in the diagnosis of PROM is to distinguish small amounts of amniotic fluid from other body fluids which may be present in the vagina. A number of non-invasive methods, based on cytologic, biochemical or biophysical differences between amniotic fluid and other body fluids have been proposed and used, but none is ideal [1–6]. Dye injection into amniotic fluid is not widely used because of its invasiveness and potential risks for the mother and fetus [1,3]. The ROM-check Membrane Immunoassay (Adeza Biochemical, Sunnyvale, California) [7] is based on the detection of fetal fibronectin in vaginal secretion. This isoform of fibronectin is present in the chorion membrane and amniotic fluid throughout gestation and its concentration in amniotic fluid is 5–10 times greater than the concentration in maternal plasma [7–9].

Insulin-like growth factor binding protein-1 (IGFBP-1) is a 25-kD protein synthesized and secreted by the fetal liver and maternal decidua [10,11]. It is present in the amniotic fluid at high concentrations from the second trimester of pregnancy until full term [12]. This study was undertaken: (1) to evaluate the diagnostic potential of IGFBP-1 measurement in cervical/vaginal secretions as an indicator of ruptured fetal membranes (ROM), (2) to compare the efficacy the IGFBP-1 measurement and the ROM-check Membrane Immunoassay in the diagnosis of ROM.

Materials and Methods

Paired samples of blood and amniotic fluid were obtained from 40 women (aged 18–41 years) undergoing amniocentesis for fetal lung maturity, bacterial staining, or Rh immunization at the Department of Obstetrics and Gynecology, Helsinki University Central Hospital, between 26 and 38 weeks of gestation. Blood was allowed to clot and then centrifuged at 3,000 rev./min for 10 min at 20°C. All samples were obtained after overnight fasting between 08:00 and 11:00 h. Morning urine samples were collected from 31 healthy pregnant women between 16 and 40 weeks of gestation. Seminal plasma was obtained from 10 male volunteers (aged 27–38 years) undergoing homologous artificial insemination or semen analysis. All samples were frozen at -20°C until assayed. A dacron swab specimen was obtained by speculum examination from the cervix of 54 women with intact membranes (confirmed by

follow up and at the time of delivery) examined for routine prenatal care between 24 and 40 weeks of gestation and from 34 women after spontaneous or artificial rupture of membranes between 24 and 41 weeks of gestation. The duration of rupture ranged from a few minutes to 8 h. The swab was immersed in a test tube containing 0.5 ml phosphate buffered saline, 0.02% azide, pH 7.4 and the specimens were frozen at -20°C until assayed.

For the comparison between the IGFBP-1 measurement and the ROM-check Membrane Immunoassay, two dacron swab specimens were taken simultaneosly at a sterile speculum examination from the vagina in 54 pregnant women between 19 and 41 weeks of gestation. One specimen was used immediately for the ROM-check Membrane Immunoassay. The other was immersed in a tube containing 0.5 ml of IGFBP-1 assay buffer for 15 s. The tubes were frozen at -20°C until assayed. Twenty pregnant women examined for routine prenatal care at 23–41 weeks had intact membranes and no contractions. Ten pregnant women (at 19–40 weeks) presented with uterine contractions with or without bleeding and 24 women (at 19–40 weeks) presented with ruptured fetal membranes as confirmed by routine clinical examination. Duration of rupture varied from 1 h to 1 week. Eight specimens were contaminated by blood. Women who had had sexual intercourse within 24 h were excluded as recommended in the instructions for the ROM-check Membrane Immunoassay [7].

The effect of vaginal proteases on IGFBP-1 was examined by incubating 1 ml of amniotic fluid with 200 μl of vaginal secretion from term pregnancy at 37°C for 0.5–18 h. The IGFBP-1 concentration in the mixture was measured before and after incubation.

A piece of fetal membranes was collected after spontaneous vaginal delivery or cesarean section from 6 women. Formalin fixed specimens were stained with immunoperoxidase staining using monoclonal antibody 6303 against IGFBP-1 as described previously [13,14].

The IGFBP-1 concentration in body fluids and in the tubes containing buffer and swab specimen were measured by a two-site immunoradiometric assay (IRMA) as described previously [15] and by a commercial immunoenzymometric assay kit (IEMA) (Medix Biochemica, Kauniainen, Finland). The two monoclonal antibodies MAb 6303 and Mab 6305 (Medix Biochemica, Kauniainen, Finland) [16] were used in both assays. In the IEMA test, IGFBP-1 from the specimen (20 μl) was sequentially bound to two monoclonal antibodies, one immobilized on microtiter plates, the other conjugated to horseradish peroxidase. After washing, substrate was added. The amount of IGFBP-1 in the specimen was proportional to the enzyme activity. Absorbance at 414 mm was measured on a microplate reader. The test can be performed in less than 2 h. The minimum detectable amount of IGFBP-1, defined by the concentration at two standard deviations above the zero was 0.4 μg/l for IRMA and 0.5 μg/l for IEMA. The intra- and interassay coefficients of variation for IEMA are 2.4–3.4% and 4.9–7.4%, respectively. The corresponding values for IRMA were 4.6–8.2% and 9.7–11.1%, respectively [15]. The results were not affected by the presence of insulin-like growth factor I or II. The IGFBP-1 values determined by IRMA correlate well with those measured by IEMA ($r = 0.98$, $P < 0.0001$). The value obtained by IEMA is 0.93 $\times$ the value obtained by IRMA + 2.6.

The ROM-check Membrane Immunoassay (Adeza Biomedical, Sunnyvale, California) is a solid-phase, immunogold assay utilizing a monoclonal antibody which specifically recognizes an epitope of fibronectin present in fetal but not adult tissues or plasma [10–12]. The assay can be performed at the bedside in 5 min. Collection and use of all specimens was approved by the Local Ethical Committee. Student's *t*-test was used for comparison of IGFBP-1 levels in different body fluids.

Results

The dilution curves of purified IGFBP-1 added to assay buffer, urine or seminal plasma are parallel with those from amniotic fluid and serum, demonstrating that the IGFBP-1 assays used in this study are valid for different body fluids (Fig. 1). The IGFBP-1 concentrations in the fluids examined are shown in Table I. In urine and seminal plasma the IGFBP-1 concentrations were significantly lower ($P < 0.001$) than those in serum. The mean (± S.E.M.) amniotic fluid: maternal serum ratio of IGFBP-1 concentrations was 407 ± 41.5 (range 95–1,250) (Fig. 2). In cervical swab specimens (immersed in 0.5 ml of sample buffer) obtained from nonpregnant women the IGFBP-1 concentration was <1 μg/l in each case and in those from pregnant women with intact membranes and not in labor, the values ranged from undetectable to 90 μg/l (median 8.6 μg/l). In cervical specimens taken less than 8 h after spontaneous or artificial rupture of fetal membranes, the IGFBP-1 concentrations were between 175 and 2×10^4 μg/l. Since the approximate dilution of vaginal secretion

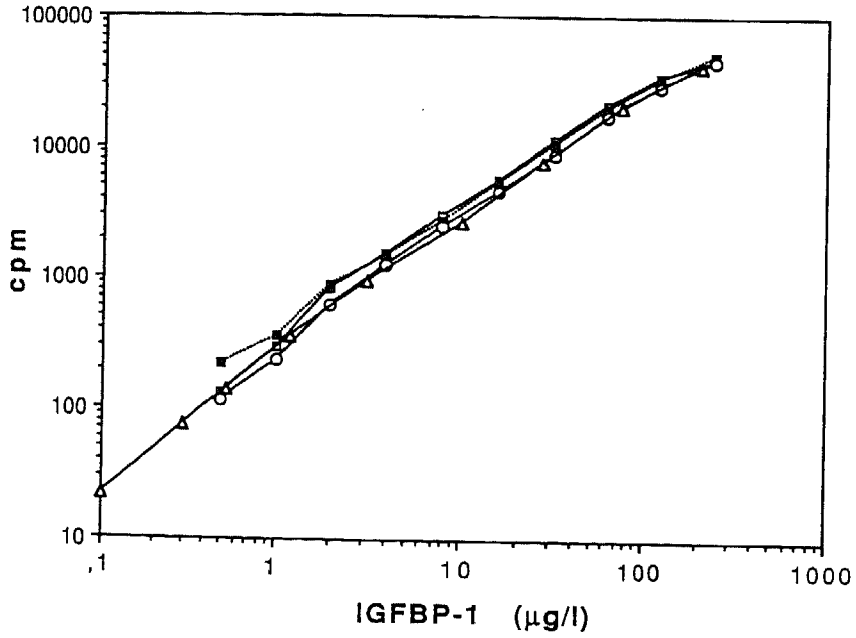

Fig. 1. Dilution curves for amniotic fluid (Δ) and purified IGFBP-1 (PP12, Behringwerke, Germany) diluted in assay buffer (O), urine (■) and seminal plasma (□) as measured by IGFBP-1 IRMA.

TABLE I

The IGFBP-1 concentrations in various body fluids as measured by monoclonal antibody-based IRMA

Body fluid	IGFBP-1 (μg/l)	
	Range	Median
Amniotic fluid	10,500–350,000	68,000
Serum	58–600	220
Urine	Undetectable, 22	Undetectable
Seminal plasma	Undetectable, 21	2

in the dacron swab specimen was 5 and the upper limit of serum (97.5 percentile) was 500 μg/l, values greater than 100 μg/l in swab specimens were interpreted as containing amniotic fluid. With this detection limit, the IGFBP-1 measurement was 100% accurate in the detection of amniotic fluid in the vagina within 8 h of rupture. After 18 h incubation with vaginal secretion, the IGFBP-1 concentration in amniotic fluid decreased in a time-dependent fashion (Table II), indicating that IGFBP-1 is degraded by proteases in the vagina.

The efficacy of the IGFBP-1 measurement and the ROM-check Membrane Immunoassay in the detection of ruptured fetal membranes were compared in 54

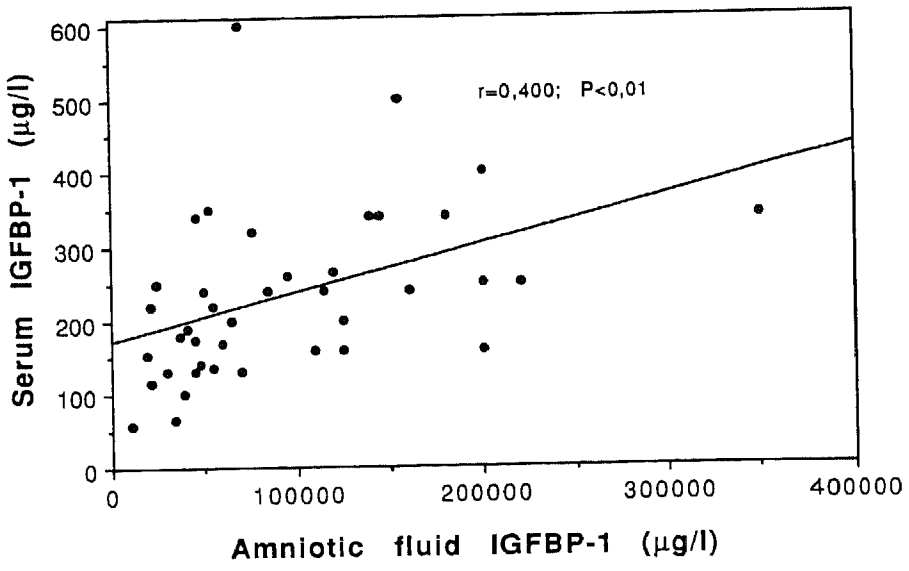

Fig. 2. The ratio of amniotic fluid and serum IGFBP-1 in paired samples obtained from 40 women at the time of amniocentesis between 26 and 40 weeks of gestation.

TABLE II

The effect of vaginal proteases on IGFBP-1 concentration in amniotic fluid

IGFBP-1% of initial	Time (h)
0	100
0.5	72
1	67
2	70
9	52
18	14

Amniotic fluid (1 ml) was incubated with 200 μl of vaginal secretion for indicated time periods at 37°C and the IGFBP-1 concentration was measured before and after incubation. The values are mean of two experiments.

women, in whom from 1 h to 1 week had elapsed since the rupture of the membranes. The data are depicted in Table III. In 24 women with ROM from 6 h to 1 week earlier, the IGFBP-1 concentration in vaginal fluid ranged from 1 to 9,000 μg/l. Six of these women, who had less than 100 μg/l (1–20 μg/l) of IGFBP-1 in vaginal secretion, all had rupture of membranes confirmed at least 12 h earlier. Two of these patients had a negative ROM-check result. In 30 patients with intact membranes, the ROM-check Membrane Immunoassay result was negative in 24 and positive in 6 cases. The one patient with intact membranes and an IGFBP-1 concentration 110 μg/l had had contractions and heavy bleeding for 2 days before sampling. In this set of patients, the IGFBP-1 level > 100 μg/l in vaginal swap specimen had a sensitivity of 75% and a specificity of 97% in the diagnosis of ruptured fetal membranes. The corresponding numbers for a positive ROM-check result were 92% and 80%,

TABLE III

Comparison of the ROM-check Membrane Immunoassay and the IGFBP-1 (IEMA) measurement in the diagnosis of ruptured fetal membranes

Patient group	No. of patients	ROM-check +	IGFBP-1 > 100 μg/l
Intact membranes, no contractions	20	4	0
Contractions, no clinical evidence of ruptured membranes	10	2	1
Ruptured fetal membranes	24	22	18

The ROM-check Membrane Immunoassay and the IGFBP-1 measurement were made parallel in vaginal swab specimens obtained from 54 women at 19–41 weeks gestation. Twenty women (23–41 weeks), who had intact membranes and no contractions, served as controls. Ten women with intact membranes (19–40 weeks) had uterine contractions with or without bleeding, and 24 women (19–40 weeks) had ruptured fetal membranes as confirmed by routine clinical means.

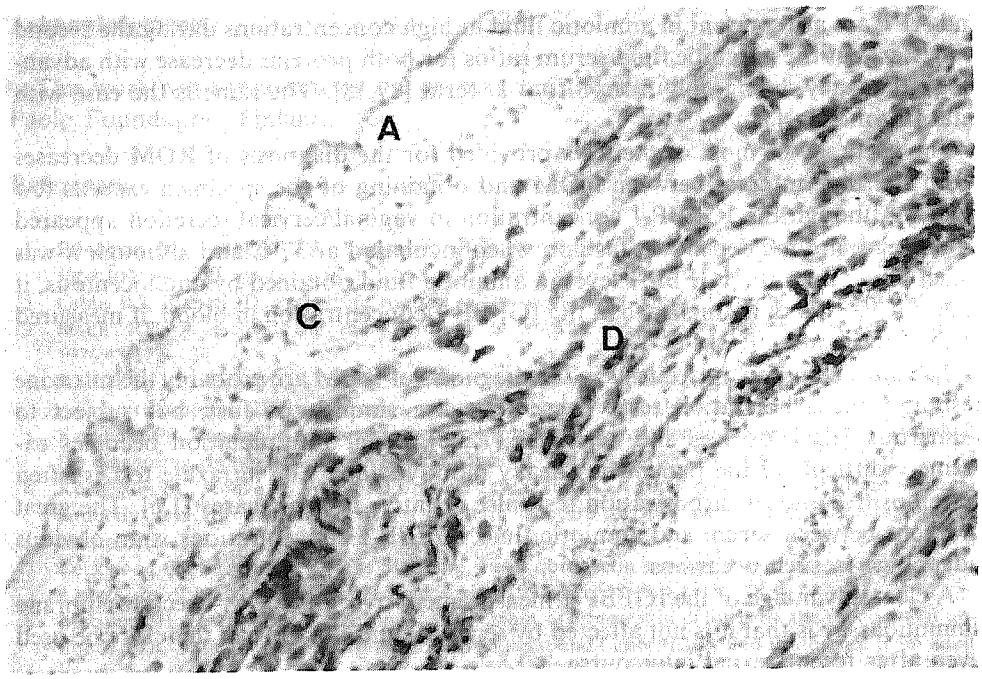

Fig. 3. Immunohistochemical staining of fetal membranes from term pregnancy with the monoclonal antibody 6303 against IGFBP-1. Note the strong specific staining in decidual cells of decidua parietalis (D) adjacent to the chorion membrane (C). (A) is amnion epithelium.

respectively. The positive predictive value was 95% for the IGFBP-1 measurement compared to 79% for the ROM-check test.

By immunohistochemical staining, IGFBP-1 was localized to decidual cells outside the chorion layer of the membranes (Fig. 3).

Discussion

PROM is a frequent diagnostic and therapeutic dilemma in obstetrics. If the patient presents with a history of a gush of fluid, the diagnosis can be made by physical examination. In uncertain cases the major contaminants of amniotic fluid which have to be considered in the diagnosis or exclusion of ruptured fetal membranes are blood, urine, cervical mucus, vaginal discharge and seminal fluid. The method reliable for demonstrating the presence of amniotic fluid in the vagina should, therefore, accurately discriminate between amniotic fluid and the nonspecific interferences. In this study, we show that tests measuring IGFBP-1 in cervical/vaginal secretion might be useful in this respect, since IGFBP-1 levels in amniotic fluid far exceed those in interfering body fluids. Furthermore, gestational age does not affect the accuracy of the IGFBP-1 measurement, since the high amniotic fluid:serum ratio of IGFBP-1 levels is retained from the second trimester to term pregnancy. Prolactin

and AFP are also present in amniotic fluid in high concentrations during the second trimester, but the amniotic fluid/serum ratios for both proteins decrease with advancing pregnancy, being only from 3 to 4 at term [17,18]. The same is the case with fetal fibronectin [7].

The accuracy of most of the tests provided for the diagnosis of ROM decreases when the time interval between ROM and obtaining of the specimen exceeds few hours. Although the IGFBP-1 concentration in vaginal/cervical secretion appeared to decrease in time dependent fashion when incubated at 37°C and although it was much lower than the IGFBP-1 level in amniotic fluid obtained by amniocentesis, it was still 10–100 times higher than the IGFBP-1 concentration in blood, if measured in few hours after ROM.

The most commonly used tests in the diagnosis of ROM are probably the nitrazine test and the arborization test. These tests are simple and fast, but subject to numerous false positives and negatives [1,2,6]. Their interpretation becomes extremely difficult, if the patient has heavy bloody show. The nitrazine test is often false positive and the arborization test false negative in those cases [1,6]. The great gradient between serum and amniotic fluid IGFBP-1 concentrations is an obvious advantage in such occasions.

A clear advantage of the IGFBP-1 measurement over the ROM-check Membrane Immunoassay is that it is not affected by seminal fluid [7] and can therefore be used even after recent sexual intercourse.

In this study, the ROM-check Membrane Immunoassay had a false positive rate of 20%, which is in agreement with previous reports [7,17]. This was significantly higher than the false-positive rate of the IGFBP-1 $>$ 100 μg/l (3%). The false-negative rate was 9% for the ROM-check test and 25% for the IGFBP-1 measurement, all the false negatives being in patients who had prolonged rupture of membranes (from 12 h to 1 week). The high false-positive rate for the ROM-check test, as well as the high false-negative rate for IGFBP-1 measurement in cases of prolonged rupture indicate that the results of even these tests need to be interpreted with caution and with consideration of patients history and physical examination.

False-positives for the ROM-check have been explained by leakage of amniotic fluid through stretched membranes. Fetal fibronectin has been localized to the chorionic layer of fetal membranes [7], and IGFBP-1 is produced by decidual cells [10,11,13,14] which are located adjacent to chorion membrane. Due to this close proximity of the cells containing fetal fibronectin and IGFBP-1, it is possible that both proteins are leaking into the vagina in occasions when the cervix dilates and chorion is detaching from the decidua in the lower uterine segment. However, no difference was found in vaginal fluid IGFBP-1 concentrations between those cases in which the ROM-check test was true negative and in which it was false positive, suggesting that only trace amount of IGFBP-1 is leaking into the vagina if the membranes are intact.

In summary, our data show that IGFBP-1 is an ideal marker of amniotic fluid and that rapid, simple tests for the measurement of this protein in vaginal secretion might have diagnostic potential in the diagnosis or exclusion of ruptured fetal membranes when used within few hours of suspected ROM.

Acknowledgments

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