

EXPERIMENTAL / LABORATORY STUDIES

Insulin-Like Growth Factor Binding Protein-I; a Rapid Detection of Amniotic Fluid Leakage after Amniocentesis

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Abstract: The aim of this study was to assess the diagnostic value of vaginal insulin-like growth factor binding protein-1 (IGFBP-I) in the detection of amniotic fluid leakage after amniocentesis.

It includes 58 healthy pregnant women who successfully underwent amniocentesis at 15-20 weeks of gestation. After amniocentesis patients were evaluated for amniotic fluid leakage at 60 min, 48 h, and 6 weeks after the procedure with speculum examination, nitrazine paper test and microscopic evidence of ferning, and were grouped into Group I (at least 2 positive tests) and Group II (others) according to the first evaluation. All patients tested for vaginal IGFBP-I.

A total of 1.1% (7/58) of patients met the Group I criteria. Vaginal bleeding and contraction occurrence was nonsignificantly higher in Group I. One false positive and one false negative result were obtained from IGFBP-I testing. The sensitivity of this test was 85%, specificity was 98%, positive predictive value (PPV) was 85% and negative predictive value (NPV) was 98%. After 48 h the sensitivity of this test was 75%, specificity was 98%, PPV was 75% and NPV was 98%. Results at the 6th week were similar to those at 48 h.

In conclusion the detection of vaginal IGFBP-I is highly predictive for amniotic fluid leakage especially in clinically doubtful cases.

Key Words: Amniocentesis, amniotic leakage, insulin-like growth factor binding protein-I (IGFBP-I)

Abbreviations: PROM: Premature rupture of membranes, IGFBP-I: Insulin-like growth factor binding protein-1, PPV: Positive predictive value, NPV: Negative predictive value

Introduction

Genetic amniocentesis is one of the most widely utilized prenatal diagnostic techniques. It presently allows the detection of chromosome abnormalities, inborn errors of metabolism, hemoglobinopathies and other biochemical disorders. However, it is also associated with some maternal and fetal complications. In the literature, vaginal leakage of amniotic fluid after amniocentesis has been reported (1).

Chronic leakage of amniotic fluid or premature rupture of membranes (PROM) is associated with increased infectious morbidity for the mother and the fetus (2,3). In the majority of cases the diagnosis is

confirmed after ascertaining the history of the patient and performing a detailed pelvic examination. However, the leakage of the amniotic fluid may be intermittent and there may be no fluid collection in the vagina, or the vaginal fluid may be contaminated with urine, semen or other secretions.

Many different tests have been developed to diagnose rupture of the fetal membranes (4). However, most of the tests lack the necessary sensitivity and specificity to diagnose PROM, especially in doubtful conditions.

A false positive diagnosis of PROM may lead to inappropriate intervention, and a false negative diagnosis may result in an increased risk of maternal and fetal

morbidity. The absence of a noninvasive gold standard for the diagnosis of membrane rupture has led to the search for alternative biochemical markers vaginal prolactin, α -feto protein, fetal fibronectin a etc. have previously been studied (5,6).

Insulin-like growth factor binding protein-I(IGFBP-I) detection has been promoted as a reliable and ideal marker to diagnose ruptured fetal membranes or amniotic fluid leakage (7-9). May initial study by Rutanen et al. reported that membrane rupture was associated with detectable IGFBP-I in vaginal secretions. IGFBP-I is a major protein in amniotic fluid and its average concentrations are \geq 400-fold higher than those in maternal serum (4).

This study was conducted to assess the diagnostic value of vaginal IGFBP-I in the detection of amniotic fluid leakage after amniocentesis.

Materials and Methods

This prospective study was carried out on 58 healthy pregnant women, recruited from parturients admitted to the perinatology department of the Zekai Tahir Burak Women's Health Education and Research Hospital. Genetic amniocentesis was performed successfully in all cases at 15–20 weeks of gestation. The indications considered for amniocentesis were as follows: advanced maternal age, a previous child with a chromosome or structural abnormality or positive family history, positive family history for neural tube defects, previous history of recurrent miscarriages and stillbirths, parents carriers of an autosomal recessive disorder diagnosable in utero and abnormal maternal serum screening results.

Exclusion criteria from the study included vaginal bleeding, uterine contractions, and the presence of any prenatal complications or multiple pregnancy.

On admission, age, parity and gestational age were registered at the time of initial evaluation. Total blood count, Rh blood type tests, physical examination and ultrasonographic evaluation were performed. The advantages and risks of the invasive procedure and information about the study were explained to all the subjects. All participants and their husbands gave their written informed consent. After ultrasonographic examination was performed to localize the placenta and fetus and to re-evaluate the gestational age approximately 15–20 cc of amniotic fluid is removed

transabdominally under sterile conditions using a 20 gauge needle.

Speculum examination was performed 60 min after the procedure. At speculum examination, vaginal pooling of fluid was registered. Nitrazine paper test and microscopic evaluation of ferning on a dried vaginal smear were performed. Patients were diagnosed as having membrane rupture if they demonstrated at least 2 of the following indices: vaginal pooling of fluid, a positive nitrazine paper test, or microscopic evidence of ferning and positive IGFBP-I testing. The actim PROM test, (Medix Biochemical OY AB, Kauniainen, Finland), was used to determine IGFBP-I in vaginal specimens. An actim PROM test was performed by obtaining a sample of vaginal secretion using a Dacron swab placed in the posterior fornix or in the cervical canal for 10 s. The swab was rinsed in a buffer tube and a dipstick was placed in the tube for approximately 20 s. The stick contains monoclonal antibodies to IGFBP-I and absorbs the extracted specimen. If the amniotic fluid contains >25 mg/l of IGFBP-I, in the extracted sample 2 blue lines will appear on the stick.

The subjects were then classified as follows: amniotic fluid leakage (+) cases were classified as Group I and amniotic fluid leakage (-) cases as Group II, according to the test results. All subjects underwent ultrasonographic examination to evaluate fetal well-being and amniotic fluid indices. All the examinations were performed by the same physician in order to eliminate interobserver sampling differences. The subjects were then re-evaluate at 48 h and at 6 weeks in terms of amniotic fluid leakage and fetal well-being.

All data were analyzed using SPSS-PC (version 11.0). For statistical analysis, Student's t test, Mann-Whitney U test and Fisher's exact test were used. Sensitivity, specificity, positive predictive values (PPVs) and negative predictive values (NPVs) of the IGFBP-I test were then calculated.

Results

Demographic characteristics of the subjects are shown in Table 1. Mean maternal age was 31.5, ranging from 18 to 43. Mean gestational age of all subjects at time of amniocentesis was 18.51 weeks (16 – 21 weeks). There were no significant differences between the 2 groups with regard to maternal age, parity or gestational age (Table 1).

Table 1. Demographic characteristics of the subjects.

Characteristics	Group I (n=7)	Group II (n= 51)	P
Age (years)	29 ± 5.6	28 ± 4.8	>0.05 ^x
Parity	1.01 ± 0.9	0.98 ± 0.8	>0.05 ^x
Gestational age (weeks)	18.8 ± 4.1	18.4 ± 3.6	>0.05 ^x
Occurrence of contractions (> 4 per hour)	1/7 (14%)	4/51 (8%)	>0.05 ^y
Vaginal bleeding	1/7 (14%)	5/51 (10%)	>0.05 ^y

Values are expressed as mean ± S.D. or number / percentages

X = Student's t test

Y = Fisher's exact test

Of the 56 subjects, 1.1% (6/56) of cases met the criteria for transient amniotic fluid leakage. The incidences of patients with vaginal bleeding and occurrence of contractions were higher in actim PROM test (+) cases than the in actim PROM (-) group. However, there were no statistically significant differences between the 2 groups with regard to these parameters. No signs of chorioamnionitis were detected in any of the patients. Only one patient from the amniotic fluid leakage (+) group delivered within 4 weeks after sampling. In this patient an obstetrical history of gestational diabetes in her previous pregnancy was recorded (Table 2).

One false positive and one false negative case were observed during the course of the study. The false positive case occurred in a patient admitted to the hospital at 21 weeks of gestation, at a later period in comparison with the other cases. The vaginal sample obtained from this woman was grossly contaminated with maternal blood, which can easily alter the result. However the false negative result detected in the other case may be attributed to inadequate sampling.

Table 2. Diagnostic values of IGFBP-I levels (just after amniocentesis).

	Actim PROM test (+)	Actim PROM test (-)	Total
Group I (n = 7)	6	1	7
Group II (n = 51)	1	50	51
Total	7	51	58

Sensitivity 85%; Specificity 98%; PPV 85%; NPV 98%

The sensitivity of the actim PROM test compared to that of other commercial tests and clinical evaluation was recorded as 85% just after the procedure. The rate of specificity was 98%, PPV was 85% and NPV was 98%.

Diagnostic values of IGFBP-I levels and the rate of amniotic fluid leakage at 48 h after the procedure are shown in Table 3. The rate of amniotic fluid leakage was 0.68% at 48 h.

The sensitivity of the actim PROM test compared to that of other commercial tests and clinical evaluation was recorded as 75% at 48 h after the procedure. The rate of specificity was 98%; PPV was 75% and NPV was 98%.

The diagnostic values of IGFBP-I with chronic amniotic fluid leakage during a check-up which performed after 6 weeks were similar to those at 48 h.

Although the incidence of chronic amniotic fluid leakage was recorded as 0.68%, neither pathology in fetal condition nor a decrease in amniotic fluid value were observed either at 48 h or 6 weeks.

Table 3. Diagnostic values of IGFBP-I levels (at 48 h after procedure).

	Actim PROM test (+)	Actim PROM test (-)	Total
Group I (n = 7)	3	1	4
Group II (n = 51)	1	53	54
Total	4	54	58

Sensitivity 75%; Specificity 98%; PPV 75%; NPV 98%

Discussion

Amniocentesis is one of the most widely used techniques in prenatal diagnosis. However it has also some maternal and fetal risks. Amniotic fluid leakage has been suggested as a possible cause of increased fetal and maternal morbidity (10,11). Tabor et al. reported a controlled trial of genetic amniocentesis in 4606 women (1). In this study amniotic fluid leakage occurred more commonly in the study group than in the control group (1.7% vs 0.4%). Amniotic fluid leakage was recorded in 1.1% of cases, consistent with the results of earlier studies in the literature.

As chronic leakage of amniotic fluid is associated with increased morbidity for the mother and the fetus, failure of diagnosis can lead to unwanted obstetric complications. If the diagnosis of amniotic fluid leakage remains uncertain using physical examination or commercial methods, the ideal method for demonstrating the presence of amniotic fluid in the vagina should be rapid and have high accuracy. Transient vaginal amniotic fluid leakage is also common after genetic amniocentesis. However, even occult rupture of fetal membranes may indicate an increased risk of preterm delivery, abortion, fetal loss and/or intrauterine infection.

Since there is no gold standard test applicable to all subjects with high accuracy, many different biochemical markers have previously been studied like fetal fibronectin, α -feto protein, prolactin and diaminoxidase (11).

Most tests diagnosing amniotic fluid leakage have limited sensitivity and specificity due to confounding factors like vaginal discharge, maternal blood loss, and cross reactions with urine or cervical mucus. A low gradient between serum and amniotic fluid has also limited the diagnostic value of some biochemical markers. Moreover, some previously used tests to detect amniotic fluid in the vagina are based on radioimmunoassay techniques, with a possible delay of the result of up to 24 h. In patients with clinically doubtful ruptured fetal membranes, this limited sensitivity and specificity become particularly apparent.

IGFBP-I detection can be easily used for the diagnosis of amniotic fluid leakage or ruptured fetal membranes in obstetrical practice. Although the precise source of amniotic IGFBP-I remains uncertain, it has been

immunohistochemically localized to the decidua, but only weak staining was observed in the amniochorionic layer. The marked gradient between blood and amniotic fluid IGFBP-I concentrations makes IGFBP-I a potential marker for the detection of amniotic fluid leakage or ruptured fetal membranes.

In the literature, several studies have indicated that IGFBP-I detection showed high sensitivity (91%–100%), specificity (83%–95%) and PPV (85%–95%) even in cases in which the diagnosis is clinically doubtful (12). In our series, it was observed that IGFBP-I detection showed a high sensitivity rate of 85%, specificity of 98%, PPV of 85% and NPV of 98%, in agreement with previous studies.

Different factors may interfere with the predictive values of IGFBP-I detection. In this study there were one false positive and one false negative case. Several reports have indicated that false positive results may be caused by contamination with grossly bloody vaginal discharge. False negative results may be caused by inadequate sampling, intra-amniotic infections or proteolytic degradation of IGFBP-I in the vagina. However Rutanen et al. reported that bloody and other secretions that may contaminate amniotic fluid in the vagina did not appear to interfere with the test results (4). In this study while the false positive vaginal IGFBP-I result was likely to be related to the presence of contamination of maternal blood, the false negative case may have been related to inadequate sampling technique.

In conclusion, the detection of vaginal IGFBP-I is highly predictive of amniotic fluid leakage or ruptured fetal membranes, especially in clinically doubtful cases. The rest is easily performed in the clinical set up and no analytical difference has been reported in the IGFBP-I assay. However, because of the possible interference with other factors, IGFBP-I detection has been suggested as a useful complement to other tests, to confirm an accurate diagnosis of amniotic fluid leakage.

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