Korean Journal of Fetal Medicine
Vol. 4 No. 4 December 2008

양막 파수의 진단에 Placental Alpha-Microglobulin-1, Insulin-like Growth Factor Binding Protein-1, Nitrazine 검사방법의 비교 분석

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Comparison of Placental Alpha-Microglobulin-1, Insulin-like Growth Factor Binding Protein-1 and Nitrazine Test in Cervicovaginal Discharge in Diagnosis of Rupture of Membranes

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Objective: To compare the value of Placental alpha-microglobulin-1 (PAMG-1), insulin-like growth factor binding protein-1 (IGFBP-1) and Nitrazine test for the diagnosis of rupture of the membranes (ROM).

Methods: We conducted a prospective study between July 2007 and April 2008. Eighty-seven pregnant women between 12 and 41 weeks of gestation with suspected ROM were included. The ruptured membranes status was defined based on clinical examination (pooling of fluid in vagina) and ultrasonographic findings (amniotic fluid index<5.0cm or amniotic fluid pocket<2.5 cm).

Results: Total 24 out of 87 patients met the above criteria for the diagnosis of ROM. The mean gestational age at the time of the testing was 28.0±6.9 weeks. Sensitivity, specificity, positive predictive value and negative predictive value of each test were as follows, respectively: Placental alpha-microglobulin-1 (54.2%, 87.1%, 61.0%, 83.1%), IGFBP-1 (87.5%, 66.1%, 50.0%, 93.2%) and nitrazine test (75.0%, 51.6%, 37.5%, 84.2%).

Conclusions: Based on our study, IGFBP-1 showed the highest sensitivity and PAMG-1 showed the highest specificity among the three tests. Positive results in both IGFBP-1 and PAMG-1 would be best to detect ROM. However, considering the cost effectiveness and diagnostic accuracy altogether, we recommend the serial testing, that is to say, the conventional nitrazine test, followed by placental alpha-microglobulin-1 test when the result of nitrazine test is positive to decrease the false positive diagnosis of ROM.

Key words: Insulin-like growth factor binding protein-1, Nitrazine test, Placental alpha -microglobulin-1, Rupture of membranes

접수일 : 2008. 12. 12.
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Rupture of the membranes (ROM) can occur at any time during pregnancy. It may happen at term prior to the onset of labor (premature rupture of membranes, PROM) or may occur before 37 weeks of gestation (preterm premature rupture of membranes, PPROM). ROM occurs in approximately 8 to 10% of all pregnancies and in 2 to 3.5% of pregnancies before 37 weeks of gestation.[1,2]

When obvious vaginal pooling or rupture is noted, diagnosis of ROM is easy, but sometimes it can be extremely difficult. One of the most conventional methods and most commonly used methods for diagnosis of ROM include nitrazine test. However, this test has high false positive and false negative rates because it can be affected by many situations like vaginitis, urine, semen or blood.[3,4,5] Measurement of amniotic fluid index (AFI) or amniotic fluid pocket (AFP) using ultrasound (US) is not always reliable because minor leakage is difficult to detect by US and presence of oligohydramnios does not always indicate ROM.[6] Placental alpha-microglobulin-1 (PAMG-1, Amnisure®) is a 34kd fetal glycoprotein that circulates in the amniotic fluid and its concentration is 1,000-10,000-fold higher in amniotic fluid than that in the cervicovaginal discharge with intact membranes. Amnisure® is a recently introduced bedside immunoassay designed to measure PAMG-1 in cervicovaginal discharge to detect ROM.[3,7,8] Insulin-like growth factor binding protein-1 (IGFBP-1, Actim PROM®) is a major protein produced by human decidua and concentrations of IGFBP-1 are about 100-1,000 fold higher in amniotic fluid compared to serum.[9,10,11] Therefore, detection of PAMG-1 or IGFBP-1 in cervicovaginal fluid may be helpful in diagnosis of ROM.

ROM is a significant cause of premature delivery and neonatal complications requiring admission.[2,12] It is difficult to balance the risk of prolonging pregnancy against the risks of acquired intrauterine and maternal infection and problems of lung development. However, incorrect diagnosis of ROM may lead to increased diagnosis of ROM and unnecessary obstetric interventions like hospitalization, antibiotics or induction of labor.[3,8] Therefore, correct and timely diagnosis of ROM is extremely important for proper management of this disorder.

The objective of the current study was to compare the value of PAMG-1, IGFBP-1 and Nitrazine test for the diagnosis of rupture of the membranes (ROM).

Materials and Methods

Between July 2007 and April 2008, a prospective, controlled trial was designed and total eighty seven healthy pregnant women with singleton baby between 12 and 41 weeks of gestation with reporting fluid loss were evaluated as with history taking, transabdominal ultrasound examination, and speculum examination. Patients with active vaginal bleeding, those with multiple pregnancies and anomalous fetuses were excluded. All the patients were subjected to Nitrazine test, IGFBP-1 test (Actim PROM®) and PAMG-1 test (Amnisure®). A sterile speculum was used to expose the cervix and the posterior fornix. Diagnosis of ROM was based on clinical examination and ultrasonographic findings. Clinical examination was considered as positive when there was increase in vaginal secretions or became watery, especially in women at the risk of preterm delivery or pooling of fluid in vagina.

Nitrazine test is a pH indicator which was interpreted as positive if the test paper turned blue. The amniotic fluid index (AFI) was assessed in four quadrants, according to the method of Phelan et al.[13] Women with AFI<5.0 cm or amniotic fluid pocket (AFP)<2.5 cm were considered to have oligohydramnios.

IGFBP-1 in cervicovaginal discharge was collected by Actim PROM® kit (Medix Biochemica, Kauniainen, Finland). The fluid was absorbed into the extraction solution and tested with the dipstick. Interpretation was performed after 5 minutes and was considered positive when two blue lines appeared on the dipstick. Two blue lines on the dipstick indicate that the sample contains IGFBP-1 above 25 μg/L and the test is positive for membrane rupture.[14]

PAMG-1 immunoassay was performed using Amni Sure

ROM® test (N-Dia Inc, New York, NY). Using the sterile speculum, a sterile Dacron swab supplied by the manufacturer was placed in the posterior fornix of the vagina. After 1 minute, the swab was removed from the vagina and was mixed into the vial containing the solvent for 1 minute. The test strip was then placed in the solvent and the result was interpreted after 5 minutes. The results were determined as positive when the control and the test lines were both present and negative when the test line was absent.[3]

After all the tests were performed and when ROM was clinically suspected, patients were managed by standard gestational age-specific clinical algorithms.

Symptoms and signs at presentation, maternal age, gestational age at inclusion, mean cervical length at presentation, gestational age at delivery and interval between testing and delivery (hours) were recorded.

For the study analysis purposes, patients were re-grouped after delivery into ROM or no ROM. The analysis was done after review of the initial testing and the clinical course. The criteria for ROM were 1) presence of watery vaginal secretions or pooling, 2) positive nitrazine test (alkaline PH), 3) oligohydramnios (AFI<8.0 cm or AFP<2.5 cm) and 4) chorioamnionitis. Diagnosis of ROM was made when the patient met two or more of the above criteria.

Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were calculated for each test. For the statistical analysis, the Student's t test and chi-square test were used. Logistic regression models were used to assess the contribution of various tests to the diagnosis of ROM and the delivery time in patients with suspected ROM. Data was processed using SPSS version 13.0. *P*-value<0.05 was considered statistically significant.

This study was approved by the Institutional Review Board of Cheil General Hospital and Women's Healthcare center and written informed consent was obtained from each volunteer prior to enrollment.

Results

After application of the diagnostic criteria that was made after delivery, 24 patients (27.6%) had ROM and 63 patients (72.4%) had no ROM. Clinical characteristics of the subjects are shown in Table 1. There was no significant difference in maternal age, gestational at initial presentation and mean cervical length at presentation when they were divided into ROM and no ROM groups. But there were significant difference in gestational age at delivery and interval between gestational age and delivery. Patients with ROM significantly delivered at earlier gestational age and had shorter interval between suspected ROM and delivery (Table 1). Symptoms and signs at initial presentation are shown in Table 2. Total 40 patients (46%) complained of vaginal leakage or showed fluid pooling in speculum examination. Others symptoms and signs included vaginal spotting (17.2%), low abdominal pain (4.6%), incidentally detected decreased AF on US (18.4%), and incidentally detected short cervix on US (13.8%).

Table 1. Clinical characteristics of the study population

Characteristics	No ROM (n=63)	ROM (n=24)	Significance
Mean age (years)	31.8±4.2	32.0±3.3	NS
GA at presentation (weeks)	28.2±6.6	27.6±7.9	NS
Mean cervical length at presentation (cm)	3.2±1.1	2.4±0.9	NS
GA at delivery (weeks)	37.9±2.7	31.8±6.1	*P*<0.01
Interval between ROM and delivery (days)	64.7±45.6	27.0±31.1	*P*<0.05

ROM: Rupture of membranes, GA: gestational age, NS: not significant.

Table 2. Symptoms and signs of the patients with suspected ROM

Symptoms or signs	Number (n=87)	Percentage
Vaginal leakage or pooling	40	46.0
Vaginal spotting	15	17.2
Low abdominal pain	4	4.6
Decrease AF on US	16	18.4
Short cervix on US	12	13.8

ROM: Rupture of membranes, AF: amniotic fluid, US: ultrasound.

The sensitivity, specificity, PPV and NPV are shown in Table 3. IGFBP-1 test showed the highest sensitivity and PAMG-1 test showed the highest specificity. Positive IGFBP-1 test can best detect ROM but with quite high false positive rate (34.9%). In contrast, the detection rate of PAMG-1 test was slightly lower than that of IGFBP-1 but the false positive rate was much lower (14.3%) in PAMG-1 test (Fig. 1). Nitrazine test showed quite high sensitivity of 75% but specificity was too low (51.6%).

In patients with suspected ROM, the main outcome measure was delivery within 14 days after each diagnostic test. Both the IGFBP-1 test and the PAMG-1 test showed statistical significance in prediction of the time interval from the application of the test to delivery (Table 4, Fig. 2).

Discussion

Diagnosis of ROM is often very difficult and history and physical examination alone are usually insufficient. Preterm premature rupture of membranes (PPROM) is a significant obstetric problem because it can cause perinatal morbidity and mortality.[15] Therefore, accurate and timely diagnosis and proper management of PPROM is important in reducing perinatal morbidity and mortality.

Diagnosis is usually made by clinical findings like watery vaginal discharge or pooling of fluid during speculum examination or suspected by decreased amniotic fluid or shortened cervical length in ultrasound examination. When the clinical diagnosis is doubtful, diagnostic tests like nitrazine test, amniotic fluid crystallization (fern test), IGFBP-1 test or PAMG-1 can be used to help the diagnosis. However, the accuracy of these tests is limited because of various confounding factors like blood, semen, alkaline antiseptics or bacterial vaginosis which may result in false positive.[3,10]

Table 3. Sensitivity, specificity, PPV and NPV of PAMG-1, IGFBP-1 and Nitrazine test in women with suspected ROM

Diagnostic tests	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PAMG-1	54.2	87.1	61.0	83.1
IGFBP-1	87.5	66.1	50.0	93.2
Nitrazine test	75.0	51.6	37.5	84.2

PPV: positive predictive value, NPV: negative predictive value, PAMG-1: Placental Alpha-Microglobulin-1, IGFBP-1: Insulin-like growth factor binding protein-1, ROM: Rupture of membranes.

Table 4. Risk of delivery in 14 days after a positive result of each test in patients with suspected ROM

Diagnostic tests	OR	CI	*P*-value
PAMG-1	3.6	1.2-10.2	0.018
IGFBP-1	3.2	1.1-8.8	0.027
Nitrazine test	1.3	0.5-3.4	0.619

OR: odds ratio, CI: 95% confidence interval, PAMG-1: Placental Alpha-Microglobulin-1, IGFBP-1: Insulin-like growth factor binding protein-1, ROM: Rupture of membranes.

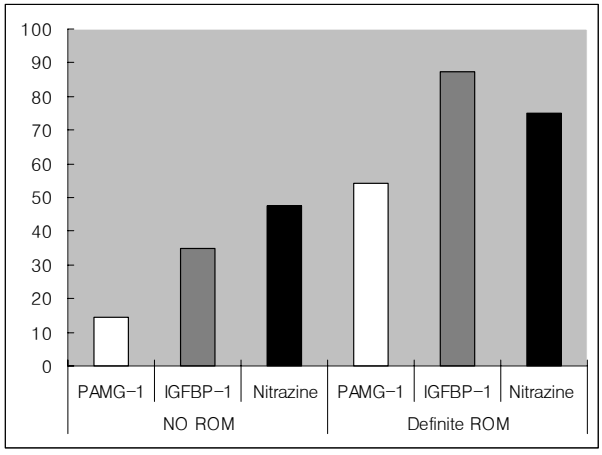

Fig. 1. Detection rates of PAMG-1, IGFBP-1 and nitrazine test in women depending on ROM status. PAMG-1: Placental Alpha-Microglobulin-1, IGFBP-1: Insulin-like growth factor binding protein-1, ROM: Rupture of membranes.

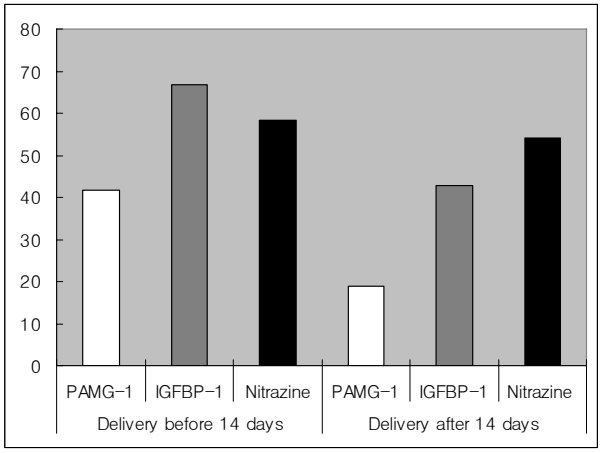

Fig. 2. Detection rates of PAMG-1, IGFBP-1 and nitrazine test in women depending on the interval between application of the test and delivery. PAMG-1: Placental Alpha-Microglobulin-1, IGFBP-1: Insulin-like growth factor binding protein-1.

One of the most accurate known diagnostic methods for PROM is ultrasound-guided amnioinfusion of indigo carmine dye followed by observation of blue fluid on speculum examination. But this method cannot be used routinely because of its invasiveness.[16]

In this study, we evaluated the value of three different kinds of diagnostic tests in diagnosis of ROM in clinically suspected cases. The AmniSure® test is a one-step immunochromatographic assay and several monoclonal antibodies are used to detect the PAMG-1 protein marker of amniotic fluid. PAMG-1 is a protein expressed by the cells of the decidual part of placenta and it was first discovered in 1970s in the Soviet Union.[7] The reasons why PAMG-1 was selected as a marker of ROM were because concentration of PAMG-1 is extremely low in cervicovaginal secretions when the fetal membranes are intact and PAMG-1 is secreted into the amniotic fluid in great quantities during pregnancy.[8] This test was developed to minimize the frequency of false positive or negative results. Background concentration of PAMG-1 is about 0.05 to 0.2 ng/mL in vaginal secretion but its concentration in amniotic fluid is around 2,000 to 25,000 ng/mL. Therefore, the presence of increased levels of PAMG-1 in cervicovaginal secretions can be a highly predictive method for diagnosis of ROM.[8]

The Actim PROM® test is a rapid bed-side test and IGFBP-1 is detected in the cervicovaginal secretions. IGFBP-1 in amniotic fluid is also increased gradually in the second trimester and remains high throughout pregnancy.[10,11]

Concentration of IGFBP-1 in amniotic fluid is around 100-1,000 times higher than he concentration in other body fluids.[11]

The nitrazine test is one of the most commonly used conventional methods in clinical practice for diagnosis of ROM because it is very simple and fast. However, the problem with this method is that it has high false-positive and negative rates and decreased accuracy.[4,5]

Previous studies have shown that detection of PAMG-1 in the cervicovaginal secretion have the sensitivity of 98.9% and specificity of 100% in the confirmation of rupture of membrane[8] and that detection of IGFBP-1 have sensitivity of 100% and specificity of 95%.[9,10,17]

In the present study, we recruited patients with suspected ROM by the report of water vaginal discharge, low abdominal pain, vaginal spotting or incidentally detected decreased AFI or shortened cervix. Patients who had positive PAMG-1 test were

3.6 times more likely to deliver within 14 days after application of the test. The test was found to have sensitivity of 54.2%, specificity of 87.1%, and 61.0% of PPV and 83.1%of NPV. Patients with positive IGFBP-1 test result were 3.2 times more likely to deliver within 14 days and sensitivity, specificity, PPV and NPV were 87.5%, 66.1%, 50.0%, 93.2% respectively. The positivity of the conventional nitrazine test did not affect the time of delivery after application of the test and sensitivity, specificity, PPV and NPV were 75.0%, 51.6%, 37.5%, 84.2%. The result of our study on PAMG-1 was different from previous study by Cousins et al.[8] because sensitivity of PAMG-1 was only 54.2% compared to 100% in the previous study. But as this test had high specificity of 87.1%, this method was proved to be good in decreasing false positive test results. The specificity of IGFBP-1 was only 66.1% compared to 95% in other previous studies.[10,17] Although the sensitivity of IGFBP-1 was highest among the three tests we evaluated, we recommend use of nitrazine test when screening for ROM because nitrazine test is much cheaper than IGFBP-1 test and it also has quite high sensitivity of 75%.

One of the limitations of the present study is that there is no 'gold standard' that can truly diagnose ROM except for an invasive test like intra-amniotic injection of indigo carmine which we could not perform because of invasiveness and inability to get an informed consent from the patients.

In conclusion, based on our study, IGFBP-1 showed the highest sensitivity and PAMG-1 showed the highest specificity among the three tests. Positive results in both IGFBP-1 and PAMG-1 would be best to detect ROM. However, considering the cost effectiveness and diagnostic accuracy altogether, we recommend the serial testing, that is to say, the conventional nitrazine test, followed by placental alpha-microglobulin-1 test when the result of nitrazine test is positive to decrease the false positive diagnosis of ROM.

Acknowledgments

AmniSure International LLC ((주) 웅비메디텍) provided all strip tests.

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「국문초록」

목적: 양막 파수를 진단하는데 이용될 수 있는 진단적 검사방법인 Placental alpha-microglobulin-1 (PAMG-1), insulin-like growth factor binding protein-1 (IGFBP-1) and Nitrazine test의 효용성을 비교해 보고자 하였다.

연구 방법: 2007년 7월부터 2008년 4월까지 제태 연령 12-41주 사이의 양막 파수가 의심되는 87명의 임신부들을 대상으로 본 연구를 진행하였다. 양막 파수의 진단은 임상적인 증상 및 초음파로 진단을 하였다.

결과: 총 87명의 환자 중 24명이 양막 파수로 진단되었으며, 검사 당시 평균 제태 연령은 28.0±6.9주였다. 각 검사 방법에 대한 민감도, 특이도, 양성예측도, 음성예측도는 각각 PAMG-1 (54.2%, 87.1%, 61.0%, 83.1%), IGFBP-1 (87.5%, 66.1%, 50.0%, 93.2%) 그리고 nitrazine test (75.0%, 51.6%, 37.5%, 84.2%)였다.

결론: 본 연구 결과에 따르면 민감도가 가장 높은 검사법은 IGFBP-1이고, 특이도가 가장 높은 검사법은 PAMG-1임을 알 수 있었다. 양막 파수를 정확하게 진단하기 위해 가장 좋은 진단 방법으로는 IGFBP-1과 PAMG-1을 동시에 시행하여 두 검사법에서 모두 양성이 나왔을 때로 진단하는 것은 제안할 수 있으나 이는 의료비용의 상승과 연관이 있을 수 있으므로, 진단의 정확성 및 비용효과적인 면을 고려해 보았을 때, 양막 파수가 의심되는 경우 민감도가 비교적 높은 nitrazine test를 먼저 시행한 후, 양성이 나온 경우 특이도가 높은 PAMG-1 검사를 시행한다면 양막 파수를 진단하는데 효과적으로 비용을 절감하면서 진단의 위양성률을 줄일 수 있을 것으로 사료된다.

중심단어: Insulin-like growth factor binding protein-1, Nitrazine test, Placental alpha-microglobulin-1, 양막 파수