Obstetrics; *Maternal- Fetal Medicine and Perinatology*

Biochemical Markers in The Vaginal Washing and Insulin-Like Growth Factor Binding Protein in The Diagnosis of Membrane Rupture

Evrim ERDEMOĞLU, Mehmet GÜNEY, Tamer MUNGAN

Isparta, TURKEY

OBJECTIVE: The aim of this study was to evaluate the diagnostic significance of IGFBP-1 applied by a dipstick method in comparison with nitrazine test and AFP/creatinine/HCG in the vaginal fluid.
STUDY DESING: Thirty-six women with definite premature rupture of membranes (PROM) and 35 women without PROM were included in the study. Speculum examination, nitrazine test and IGFBP-1 detection by a rapid strip test and the analyses of AFP, HCG, and creatinine were performed.
RESULTS: Sensitivity of IGFBP-1 test and nitrazine test was estimated to be 97%. Cut-off values for AFP (21 IU/l), creatinine (0.24 mg/dl) and HCG (702 mIU/ml) were determined by ROC analysis. Regression analysis revealed IGFBP-1 ($p<0.01$) as the most accurate test. Combination of AFP and creatinine was beneficial in discriminating membrane rupture ($p<0.01$). Likelihood ratios of PROM test, AFP, and combined AFP-creatinine tests were all of the same order of magnitude (≈34,≈0,05) and likely to be clinically important but not significantly different from each other in their clinical performance.
CONCLUSION: IGFBP-1, AFP and combination of AFP-creatinine are accurate tests, holding immense importance with regard to clinical performance.

(Gynecol Obstet Reprod Med;13:2 76-79)

Key Words: Premature rupture of membranes; Insulin-like growth factor binding protein-1; Nitrazine; Diagnosis; Alfa-fetoprotein; Creatinine; Human Chorionic Gonadotropin

Premature rupture of membranes (PROM) is a major factor that is conducive to complicating an important proportion of pregnancies, thereby significantly increasing fetal-maternal morbidity and mortality.[1] The diagnosis of PROM is unconvincing and inaccurate if the leakage of amnion through cervix is not observed during the physical examination. The accuracy of the traditional tests remains controversial. As traditional tests have high false positive and negative rates, new methods of diagnosing PROM seem essential. As would the false positive results cause unnecessary intervention increasing morbidity and mortality, so would the false negative results due to missed and probably inaccurate diagnosis.

It has been reported that insulin-like growth factor binding protein-1 (IGFBP-1), alpha-fetoprotein (AFP), human chorionic gonadotropin and creatinine in the vaginal fluid are of diagnostic significance in PROM.[1,2] We studied the accuracy of IGFBP-1 and the biochemical markers in comparison with the traditional nitrazine test.

Department of Obstetrics and Gynecology Faculty of Medicine University of Süleyman Demirel Isparta, Turkey

Corresponding Author: *Evrim Erdemoğlu*
Kıbrıs Sok. Elçi Apt. 25/7
Aşağıayrancı, 06690, Ankara, Turkey

Submitted for Publication: 16.05.2007
Accepted for Publication: 29.08.2007

Material and Metods

Seventy-one single pregnancies of 20-42 gestational weeks were included in the study conducted during 2002 and 2004. The study has received approval by the Institutional Ethical Committee. Prior to participation in the study, the patients were asked for informed consent. Thirty-six patients out of 71 recruited for evaluation had definitely underwent PROM, which was diagnosed by the amnion leakage observed through the cervix, while rest of the 35 participants were healthy controls. All patients underwent speculum examination nitrazine test and their IGFBP-1, AFP, HCG and creatinine in the vaginal fluid were evaluated. Nitrazine is a test that depends on the pH measurement of the sample. Vaginal pH is supposed to be in the range of 4.5-5.5 in healthy women. However, contamination with amniotic fluid increases the pH, turning the color of the nitrazine paper to blue.

The IGFBP-1 was detected by a dipstick method- PROM test (Medix Biochemica, Kauniainen, Finland). The test involves obtaining the sample from the posterior vagina with a Dacron® swab or from the cervix in case no leakage is visible. The swab is allowed to absorb the fluid from the vagina, followed by extracting the sample using a buffer solution. The swab is then removed and the absorbing end of the dipstick is placed in the extracting buffer solution. The PROM test is based on the use of two monoclonal antibodies to human IGFBP-1 and immunochromotography. The dipstick embod-

ies a line with immobilized anti-mouse immunoglobulins, which attracts labeled blue latex particles. The single blue line result is obtained irrespective of the presence of IGFBP-1. If IGFBP-1 is present, it binds to antibody-labeled blue latex particles, which are bound in a second line on the dipstick. The two blue lines (immobilized and catching) on the dipstick indicate that the sample contains a minimum of 25-50µgr/l of IGFBP-1.

Biochemical markers were studied in vaginal washing of 3 cc of physiological serum. The samples were centrifuged at 3000-4000 rpm/minute. Creatinine was analyzed with Jaffe method (based on picric acid) by a colorimetric test (Roche diagnostic GmbH, D-68298, Manheim, Germany) in the Roche Hitachi Modular system. Color intensity of creatinine-picric acid has been shown to be directly proportional to the creatinine concentration and therefore, it can be measured photometrically. AFP and HCG were studied by chemiluminens method in IMMULITE® 2000 (DPC, Biermann, Germany). Results of biochemical markers were available in 2-4 hours.

Statistical Analysis

Student's t-test and Mann–Whitney rank sum test were employed to compare continuous variables. The chi square test and risk estimation were used to evaluate the non-continuous data. The sensitivity, specificity and accuracy of each test were analyzed. Spearman and Pearson correlation tests were applied to reveal correlation. ROC analysis was performed for biochemical markers to evaluate diagnostic significance and to determine the cut-off values. Discriminant analysis was also performed with biochemical markers. Logistic regression and linear regression were studied for the predictive ability of the test on the membrane rupture and for the selection of the most accurate model. Prevalence, negative and positive likelihood ratios were calculated. An alpha level of 0.05 was considered statistically significant in all of the analyses. The statistical package for social sciences for windows version 11.0 and Minitab 13.2 were used for statistical analyses.

Results

Mean age, gestational age and gravida of the patients were estimated to be 25.5±5.4 years, 30.8±4.5 weeks and 2.4±1.6 respectively and no significant differences between the groups were observed (p>0.05). The prevalence of PROM in the study was 50.7%. The sensitivity of nitrazine test was shown to be 97%, the specificity was 16% and the accuracy was 56%. The sensitivity, specificity and accuracy of PROM test were 97%. Whereas a positive PROM test qualified to be a significant determiner of the PROM status (p<0.001), nitrazine test did not (p>0.05) (Table 1).

Table 1. The tests used in the diagnosis of PROM. The most accurate test is found to be PROM test followed by AFP in the vaginal washing.

	Accuracy	OR (95% CI)
Nitrazine test	56%	6,8 (0,7-59)
PROM test	97%	1190 (71-19799)
AFP cut-off 21 IU/l	95%	577 (50-6672)
Creatinine cut-off 0,24 mg/dl	88%	82 (15-438)
HCG cut-off 702 mIU/l	76%	22 (4-109)

r: correlation coefficient
OR: odds ratio
CI: 95% confidence interval

The ROC curve analysis yielded that the most significant test to diagnose PROM was AFP, followed by creatinine and HCG (Figure 1). The best cut-off value of AFP was 21 IU/l with a sensitivity and false positivity of 94% and 2.8% respectively (p<0,001). Contrarily, creatinine with a cut-off value of 0.24 mg/dl had a sensitivity and false positivity of 83% and 5.6% respectively (p<0,001). Moreover, HCG with a cut-off value of 702 mIU/ml had a sensitivity and false positivity of 57% and 5.6% respectively for the diagnosis of PROM (p<0,001) (Table 1).

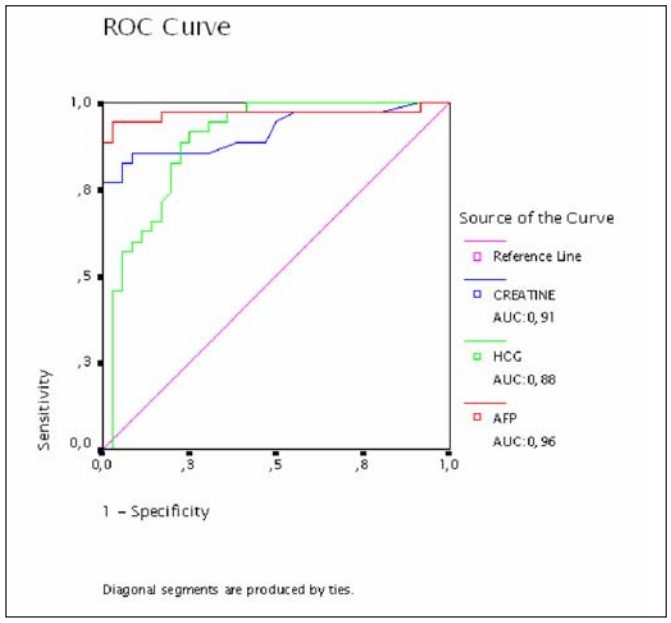

Figure 1. Assessment of biochemical markers in the vaginal fluid in the diagnosis of PROM
AFP^{AUC} > $Creatinine^{AUC}$ > HCG^{AUC}
AUC: Area under the curve

All of the diagnostic tests depicted a diagnostic predictive value, except for the nitrazine test. The most significant predictor was PROM test (p<0.001), while AFP contributed to PROM test as the second single significant predictor (p=0.001). Analysis of biochemical markers in the regression model and discriminant analysis of the diagnostic tests are shown in Table 2. The most precise model of diagnosing PROM by biochemical markers was found to be a combination of AFP and creatinine.

Table 2. Regression analysis and discriminant analysis of the biochemical predictors of PROM. The most precise prediction model seems to be a combination of AFP and Creatinine, which fits to data. AFP: alfa-fetoprotein, HCG: human chorionic gonadotropin. Cr: Creatinine.

Number of Tests Included	Diagnostic Biochemical Tests			Proportion of correct estimates PROM	Proportion of correct estimates of intact membranes	Accuracy
	AFP	Cr	HCG			
1	√			94%	94%	94%
1		√		77%	94%	85%
2	√	√		94%	97%	95%
2	√		√	91%	94%	93%
3	√	√	√	94%	97%	95%

√: Predictor included into the model.

PROM test results strongly correlated with AFP (r=0.91) and creatinine (r=0,78). However, there was a weaker correlation between PROM test and HCG. Nitrazine test did not correlate with either of the diagnostic tests.

Vaginal discharge was evident in 56.3% of cases. There was vaginal bleeding and history of coitus within 48 hours in 12.7% and 17% of patients, respectively. We analyzed the effect of these situations on the test results of women not manifesting PROM. Nitrazine test results had a significant effect due to vaginal discharge (p<0.01). However, PROM test and biochemical markers did not show any effect. Furthermore, neither bleeding nor history of coitus affected the results of the tests.

Clinical performance of the diagnostic tests was analyzed by the likelihood ratio test. PROM test, AFP>21 IU/l and combination of AFP-creatinine generated large, conclusive changes from pre-test to post-test probability. Clinical performance of these tests was clinically important, as the likelihood ratios were all of the same order of magnitude (Table 3), but were not significantly different from each other.

Table 3. Comparing PROM test, AFP, and combined AFP-Creatinine tests the likelihood ratios are all of the same order of magnitude and likely to be clinically important but not significantly different from each other in their clinical performance. Nitrazine and HCG make small changes in the probability.

Diagnosis of PROM by	Likelihood Ratio Test	
	+	-
PROM test	34,97	0,02
AFP>21 IU/l	33,94	0,05
Creatinine>0,24mg/dl	14,91	0,18
HCG>702 mIU/l	10,28	0,45
AFP * Creatinine	33,05	0,05
Nitrazine	1,16	0,17

Discussion

The diagnosis of PROM is uncertain if the amnion leakage through cervix cannot be observed in the physical examination as there is no single reliable test in practice.[3] Even occult membrane rupture or transudation of the amniotic fluid into the vagina may indicate an increased risk of fetal infection and preterm delivery. Therefore it is essential that the ideal method of detecting amniotic fluid in the vagina must be rapid and accurate. We analyzed the accuracy of new diagnostic methods, compared them with each other along with the combination of the tests in order to document the most accurate, practical way of diagnosing PROM.

Nitrazine test is the most common procedure used to diagnose PROM but not without short comes; it has a high false negative and false positive rate.[1,4,5] Nitrazine test results are unreliable in patients with vaginal discharge/ bleeding/ semen.[6,7,8] Vaginal examination with an alkali solution also increases the false positive rate of nitrazine test. Brian JM et al. found that nitrazine test has high false positive rates and the test is affected by blood and semen, although they collected the vaginal fluid with a special pouch.[7] In our study, sensitivity of nitrazine test was estimated to be 97% but the specificity and accuracy were of limited value to be considered as effective diagnostic test.

IGFBP-1 is the major protein of amniotic fluid.[8] The sensitivity and the specificity of the IGFBP-1 detection were reported to be 75-100% and 90-100%, respectively.[5,8,9] This variation was due to different cut-off values used in the studies. We used a dipstick test to study IGFBP-1, which can detect at a minimum of 25-50µgr/l of IGFBP-1 in the vaginal secretions. Takeyoshi et al. reported that the optimal cut-off concentration should be in the range of 20.1-148.4µgr/l.[8] The immunochromotographic dipstick method can be easily performed, furnishing reliable results in a few minutes. Darj et al. reported the sensitivity and specificity of the IGBP-1 detection by dipstick method as 95% and 93%, respectively.[2] Both sensitivity and specificity of the test in our study was 97%.

False positive results of the IGFBP-1 test may be due to stretching of the fetal membranes.[5] The PROM test undergoes a significant effect due to sample collection; a brief duration of holding the Dacron swab in the cervix and the buffer solution may cause erroneous results. However, PROM test results are not affected by infection/blood/semen.[5,6,8,9]

The higher concentration of AFP, HCG, creatinine and other substances in the amniotic fluid makes them suitable candidates for the diagnosis of membrane rupture. The accuracy of the biochemical markers varies with the method used; it has been reported that the detection of AFP by latex agglutination test was of limited value.[10,11] Kishida et al. developed a kit for rapid detection of AFP in the vaginal secretions. The sensitivity and the specificity of this kit utilizing anti-AFP monoclonal antibody is 96.9% and 92.9%, respectively.[1] In our study, all of the biochemical markers were significant to diagnose PROM. However, creatinine and AFP were superior to HCG. Creatinine, with a good discriminating ability, has the advantage of being cheap compared to the others. Li H-Y et al. reported that creatinine and AFP were more accurate than HCG [12] and the sensitivity and specificity of the biochemical markers were similar to our results. Contamination of the specimen may affect the accuracy of the biochemical markers in the vaginal fluid.[11] However, contamination had no effect on AFP, creatinine and HCG tests and the most accurate way of diagnosing PROM by these markers appeared to be a combination of AFP and creatinine in our study.

The 95% confidence intervals of accuracy of tests overlap by a large margin. We therefore performed likelihood ratio test to assess clinical performance of the diagnostic tests. Evaluation of predictive values was hazardous in this and other studies because of the dependence of predictive values on population prevalence. Though PROM test was found to be the most accurate single test, the likelihood ratios of AFP and combined AFP-creatinine could be held as alternatives. While creatinine with an accuracy of 88% generates moderate shifts in the pre-test to post-test probability, nitrazine and HCG should not be considered as applicable in the clinic.

Nitrazine test is affected by various factors (vaginal discharge, coitus), which also play a significant role in the etiology of PROM. False positive and false negative rates are high and it seems unreliable in the clinical practice. PROM test, AFP and combination of AFP-creatinine are accurate tests and clinical performance of these is important for the diagnosis of PROM. Diagnosing PROM by HCG levels in the vaginal fluid was not found to be applicable in this study. Comparing IGFBP-1 dipstick method with others, the only advantage observed was of PROM test that it is a rapid bedside test. However, this method may prove costly in third world countries and the application of AFP and creatinine could be an alternative method.

References

1. Kishida T, Yamada H, Negishi H, Sagawa T, Makinoda S, Fujimoto S. Diagnosis of premature rupture of the membranes in preterm patients, using an improved AFP kit: comparison with ROM-check and/or nitrazine test. Eur J Obstet Gynecol 1996;69:77-82
2. Darj E, Lyrenas S. Insulin-like growth factor binding protein-1, a quick way to detect amniotic fluid. Acta Obstet Gynecol Scand 1998;77:295-7
3. Jain K, Morris G. A clinical study to evaluate the usefulness of the MAST test in diagnosing pre-labour rupture of membranes. J Obstet Gynecol 1998;18(1):33-6
4. Rochelson BL, Rodke G, White R, Bracero L, Baker DA. A rapid colorimetric AFP monoclonal antibody test for the rupture of the membranes. Obstet Gynecol 1987; 69:163-6
5. Rutanen E-M, Kärkkäinen TH, Lehtovirta J, Uotilla JT, Hinkula MK, Hartikainen A-L. Evaluation of a rapid test for insulin like growth factor binding protein-1 in the diagnosis of ruptured fetal membranes. Clin Chim Acta 1996; 253: 91-101.
6. Kärkkäinen T, Rutanen E-M. Development of rapid immunochromotographic test for detection of premature rupture of fetal membranes. Kliin lab1998;3:176-9
7. Brien JM, Mercer BM, Sibai BM.The use of a modified vaginal pouch for the diagnosis and management of premature rupture of the membranes. Am J Obstet Gynecol 1995;172(5):1565-7
8. Kubota T, Takeuchi H. Evaluation of Insulin-like growth factor binding protien-1 as a diagnostic tool for rupture of the membranes. J Obstet Gynecol Res 1998;24(6):411-7.
9. Lockwood CJ, Wein R, Chien D, Ghidini A, Alvares M, Berkowiz RL. Fetal membrane rupture is associated with the presence of insulin-like growth factor binding protein-1 in vaginal secretions. Am J Obstet Gynecol 1994; 171: 146-50
10. Hjertberg R, Belfrage P, Eneroth P. Latex agglutination test for alfa-fetoprotein in the diagnosis of premature rupture of the amniotic membranes. Acta Obstet Gynecol Scand 1987;66:437-9
11. Gaucherand P, Guibaud S, Rudigoz C, Wong A. Diagnosis of premature rupture of the membranes by the identification of alpha-feto-protein in vaginal secretions. Acta Obstet Gynecol Scand 1994;73:456-9
12. Li H-Y, Chang T-S. Vaginal Fluid creatinine, human chorionic gonadotropin and alfa-feto protein levels for detecting premature rupture of the membranes. Chin Med J 2000; 63:686-90.