Özgün Araştırma / Original Article

Jinekoloji - Obstetrik ve Neonatoloji Tıp Dergisi
The Journal of Gynecology - Obstetrics and Neonatology

Comparison of PAMG-1 versus IGFBP-1 and Nitrazine in Diagnosis of Rupture of Membranes

Membran Rüptürü Tanısında PAMG-1 ile IGFBP-1 ve Nitrazinin Karşılaştırılması

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ÖZ

Amaç: Bu çalışmada, rüptür durumu bilinen ve bilinmeyen kadınlarda membranrüptürünün (ROM) öngörülmesinde insülin benzeri büyüme faktörü bağlanma proteini-1 (IGFBP-1) ve nitrazin testi ile plasental alfa mikroglobülin-1 (PAMG-1) karşılaştırıldı.

Gereç ve Yöntemler: Bu prospektif çalışmaya, Eylül 2006 ve Mayıs 2007 tarihleri arasında Okmeydanı Eğitim ve Araştırma Hastanesi, Kadın Doğum polikliniğine başvuran, rüptür durumu bilinen ve bilinmeyen, 24 ila 42. gebelik haftasında olan toplam 90 hasta alındı. Rüptür durumu bilinen hastalarda tanı konvansiyonel yöntemler ile konurken, rüptür durumu bilinmeyen hastalara PAMG-1, IGFBP-1 ve nitrazin testi yapıldı. Hastalar üç gruba ayrıldı: ROM bulgusu olmayan Grup 1 (n=30), tipik spontan ROM bulgusu olan Grup 2 (n=30) ve ROM şüphesi olup, konvansiyonel yöntemler ile tanı konulamayan Grup 3 (n=30). PAMG-1, IGFBP-1 ve nitrazin testlerinin performans ölçümü yapıldı ve karşılaştırıldı.

Bulgular: Hastaların ortalama yaşı 26.1 yıl idi. Nitrazinin duyarlılığı, özgüllüğü, pozitif prediktif değeri (PPV) ve negatif prediktif değeri (NPV) sırasıyla %94.4, %50.0, %73.9 ve %85.7 idi. Bu değerler IGFBP-1 testi için sırasıyla %77.8, %83.3, %87.5 ve %71.4 iken, PAMG-1 için sırasıyla %94.4, %100, %100 ve %92.3 idi. Bu sonuçlara göre, PAMG-1 genel popülasyonda ve ROM durumu bilinmeyen hasta grubunda en yüksek tanı değerine sahipti.

Sonuç: Bu çalışma, IGFBP-1 ve nitrazinin bilinmeyen ROM tanısında kullanışlı olmadığını ve PAMG-1 testinin rüptür durumu bilinen ve bilinmeyen hastalarda daha doğru bir biyobelirteç olduğunu göstermektedir. Bununla birlikte, PAMG-1 rüptür durumu bilinmeyen hastaların ROM tanısında basit ve etkili bir araçtır.

Anahtar Kelimeler: Erken membranrüptürü, plasental alfa mikroglobülin-1, insülin benzeri büyüme faktörü bağlanma proteini-1, nitrazin.

ABSTRACT

Objective: This study aims to compare the placental alpha microglobulin-1 (PAMG-1) versus insulin-like growth factor binding protein-1 (IGFBP-1) and nitrazine test for the prediction of rupture of membranes (ROM) in women with known and unknown rupture status.

Methods: This prospective study included a total of 90 patients with known and unknown rupture status who were between 24 and 42 weeks of gestation at Okmeydani Training and Research Hospital, Obstetrics and Gynecology outpatient clinics between September 2006 and May 2007. In patients with known rupture status, diagnosis was based on conventional methods, while the PAMG-1, IGFBP-1, and nitrazine tests were performed in patients with unknown rupture status. The patients were divided into three groups as Group 1 (n=30) without any signs of ROM, Group 2 (n=30) with typical signs of spontaneous ROM, and Group 3 (n=30) with symptoms suggestive of ROM who were unable to be diagnosed using conventional methods. Performance metrics of PAMG-1, IGFBP-1, and nitrazine tests were calculated and compared.

Results: Of the patients, the mean age was 28. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of nitrazine was 94.4%, 50.0%, 73.9%, and 85.7%, respectively. These values were 77.8%, 83.3%, 87.5%, and 71.4% for the IGFBP-1, respectively and 94.4%, 100%, 100%, and 92.3% for the PAMG-1, respectively. Accordingly, the PAMG-1 showed the highest diagnostic value in both the overall population and those with an equivocal status of ROM.

Conclusion: Our study results suggest that IGFBP-1 and nitrazine are not suitable for the diagnosis of unknown ROM and the PAMG-1 is a more accurate biomarker in patients with both known and unknown status of ROM. In addition, it is a simple and effective tool for the diagnosis of ROM in patients with unknown rupture status.

Keywords: Premature rupture of membranes, placental alpha microglobulin-1, insulin-like growth factor binding protein-1, nitrazine.

INTRODUCTION

Premature rupture of membranes (PROM) is defined as rupture (amniorrhexis) of fetal membranes before the onset of labor (1, 2). It can occur at term, defined as rupture after 37 weeks of gestation (GA), or in preterm patients, and preterm premature rupture of membranes (pPROM), occurring before 37 weeks of gestation. Currently, management of PROM is considered one of the most difficult dilemmas in the practice of obstetrics and the risks of prolonged latency must be balanced against the threat of prematurity, both of which carry significant risks associated with fetal mortality, morbidity, and maternal complications.

Preterm premature rupture of membranes occurs in 2 to 3% of all pregnancies and contributes to 30 to 40 % of pretermbirths (3), and more than double that in pregnancies with multiple gestations, leading to 20 to 40% of preterm births (4-7). During the latency period from rupture to delivery, the patient is more likely to experience complications associated with infections, such as maternal chorioamnionitis and fetal distress, respiratory distress syndrome, and fetal neonatal sepsis. In addition, pPROM is associated with ahigher risk of cord compression, anhydramnios, prematurity, placental abruption, and amniotic infection syndrome.Also, pPROM<20

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Başvuru tarihi: 19.09.2018
Kabul tarihi: 21.09.2018

weeks of gestation may cause limb/face deformities, severe lung hypoplasia, and dry lung syndrome which is associated with a high mortality and long-term pulmonary complications (8-9-10-11). An epidemiologic analysis showed that infections of the genital area such as Group B streptococcus, chlamydia trachomatis, Neisseria gonorrhea, and anaerobic bacterial vaginosis, and colonization of genital mycoplasma increased the risk of incidence of PROM (1,12-13), indicating that one of the major threats to the patient is often already present from the moment of rupture.

Currently, the prevention of ROM is not a practical approach for reduced PROM and pPROM rates, mainly due to two reasons. First, many risk factors are related to the patient history or lifestyle, such as smoking, malnutrition, or prior multiple pregnancies (1,14,15,16,17). Second, the exact pathogenesis of ROM has not been elucidated yet (13) and, therefore, no preventive measures have been agreed. To date, only a few hypotheses have been proposed which suggest that sufficient endurance of the surface tension of the fetal membranes depends on a balanced synthesis and degradation of extracellular matrix components. In this context, reduction of the collagen content in membranes, changes in the structure, and increasing collagenolytic activity can be associated with PROM (18). More importantly, a timely and accurate diagnosis is critical for the management process.

Nitrazine, which is a pH indicator dye, is commonly used to evaluate PROM in pregnant women. However, it has high false negative and false positive rates (19). In addition, nitrazine test results are in reliable in patients with vaginal discharge or bleeding (19-21). In a comparative study, Gaucherand et al. (18) reported that the sensitivity and specificity of nitrazine test was 90.7% and 77.2%, respectively for the diagnosis of PROM.

Insulin-like growth factor binding protein-1 (IGFBP-1) is the major protein of amniotic fluid. The immunochromotographic dipstick method can be easily performed, yielding reliable and quick. In a study, Darj et al. reported the sensitivity and specificity of the IGBP-1 detection by dipstick method as 95% and 93%, respectively (22).

Placental alpha microglobulin-1 (PAMG-1) is also an ideal biomarker for PROM, as its concentration in the amniotic fluid is 1,000 to 10,000-fold higher than that in the cervicovaginal discharge with intact membranes (23). Also, it is not present in urine or semen and at low levels in the maternal blood, which reduces the probability of inaccurate results in the presence of other fluids (23).

Although several studies have investigated the diagnostic value of these biomarkers, there is no head-to-head study of these tests in patients with known and unknown ROM status. In the present study, therefore, we aimed to compare the PAMG-1 versus IGFBP-1 and nitrazine test for the prediction of ROM in women with known and unknown rupture status.

MATERIAL AND METHODS

Study Population

This prospective study included a total of 90 patients with known and unknown rupture status who were between 24 and 42 weeks of gestation at Okmeydani Training and Research Hospital, Obstetrics and Gynecology outpatient clinics between September 2006 and May 2007. Inclusion criteria were as follows: admission to hospital during the second or third trimester of a singleton pregnancy without any history of preterm labor or pPROM; having no uterine pathology or malformation; having normal ultrasound measurements of fetal biometry as of the last menstrual period. Exclusion criteria were as follows: having a diagnosis of placenta previa or having significant amount of vaginal bleeding. All patients were informed about the study in detail and a written informed consent was obtained from each patient. The study (14.08.2006 date 17 number) protocol was approved by the Okmeydani Training and Research Hospital Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

In patients with known rupture status, diagnosis was based on conventional methods, while the PAMG-1, IGFBP-1, and nitrazine tests were performed in patients with unknown rupture status. The patients were divided into three groups as Group 1 (n=30) without any signs of ROM, Group 2 (n=30) with typical signs of spontaneous ROM, and Group 3 (n=30) with symptoms suggestive of ROM including vaginal discharge, vaginal fluid drainage, and perineal wetness who were unable to be diagnosed using conventional methods. Then Group 3 was further divided into two subgroups as Group 3a (negative clinical findings for ROM) and Group 3b (positive clinical findings for ROM) and were followed until delivery.

Procedures

Data including medical history, demographic characteristics, maternal age, gravidity, and parity were recorded. All patients who were admitted after GA Week 28 underwent non-stress test. All patients received a sterile speculum examination and were evaluated for liquid pooling, cervical fluid drainage during the Valsalva maneuver, cervical dilation, effacement, umbilical cord prolapse, cervicitis, vaginitis, blood, urine, meconium, and the presence of semen.

Samples for the nitrazine (Lackmuspapier rat™), PAMG-1 (AmniSure ROM Test, AmniSure International LLC, a QIAGEN Company, Boston, MA, USA), and IGFBP-1 (ActimProm, MedixBiochemica OY, Espoo, Finland) tests were obtained according to the manufactures' instructions. Nitrazine test samples were collected with the litmus paper tape for 10 sec from the posterior fornix of the vagina. A positive result was recorded, when the color band changed from dark pink to green or blue. The IGFBP-1 test sample was collected by placing a sterile Dacron swab in the posterior vaginal fornix for 10 sec. The swab was, then, washed for 10 to 15 sec in the sample extraction solution. The lower end of the test strip was placed into the test solution for 20 to 40 sec and, then, removed and placed horizontally for 5 to 10 min. The test result was evaluated after five minutes. A positive result was interpreted, if two blue lines appeared on the strip, while a single blue line indicated a negative result, and the test application was deemed invalid if no lines appeared. As IGFBP-1 levels are affected by vaginal protease activity, occurrence of ROM and the time interval to collection of the sample is correlated. Hence, the amniotic fluid samples of ruptured membrane in patients in Group 2 were collected within eight hours after their arrival hospital.

The PAMG-1 test sample was obtained by inserting the test kit's sterile polyester swab into the patient's vagina 5 to 7 cm and holding it in place for one min. The polyester swab was, then, placed into the vial containing extraction buffer and washed for one minute. After the removal of the swab from the solution, the test strip was inserted into the vial such that the blue arrows pointed downward into the vial. The test strip was removed immediately after two lines appeared, or after a minimum of five min and a maximum of 10 min. The test strip was placed on a clean, dry, flat surface to read the results. Two lines indicated a positive result, while only one line indicated a negative result. No lines indicated an invalid result.

The patients in Group 1 and Group 2 were managed according to the established protocol of the hospital. For the patients in Group 3, the presence of vaginal pH via nitrazine test, as well as PAMG-1 and IGFBP-1 in the cervicovaginal fluid was determined. In case of discrepant results, a diagnosis was based on the two tests with the same results.

The test result was deemed a true positive (Group 3b), if term patients went into labor within 48 hours, if preterm patients delivered within two weeks, or if any patient developed an infection after sample collection. Cases presenting with maternal fever in patients above 38°C, leukocytosis (>20,000/mm3), uterine motion tenderness, and maternal and fetal tachycardia were classified as infected. All other cases were deemed a true negative (Group 3a).

Sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of the PAMG-1, IGFBP-1, and nitrazine tests for the diagnosis of ROM at the time of diagnosis and after delivery were calculated.

STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows version 10.0 software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were expressed in mean ± standard deviation (SD), median (min-max) or number and frequency. The Student's t-test, Mann-Whitney U test, Kruskal-Wallis test, one-way analysis of variance (ANOVA), Fisher's exact test and chi-square test were used for the statistical analysis of the data. Sensitivity, specificity, NPV, PPV values were calculated. A p value <0.05 was considered statistically significant.

RESULTS

All ROM-positive patients at term delivered within 48 hours or developed an infection, while all ROM-positive preterm patients delivered within two weeks or developed an infection. There was no significant difference in the standard clinical assessment of ROM at the time of presentation compared to the ultimate determination of the rupture status after follow-up. Therefore, the metrics calculated at the time of presentation were determined to be accurate.

Table 1 shows baseline demographic characteristics of the patients. The mean age of the patients was similar among all groups. While the mean GA was similar in Groups 2 and 3, the mean GA of Group 2 was significantly

higher than the other two groups. Similarly, Group 2 had a significantly higher effacement and dilation values. The patients in the suspected ROM group had significantly higher rates of vaginal infection than the other groups.

Table 1. Demographic and clinical characteristics of patients

	Group 1 (n=30)		Group 2 (n=30)		Group 3 (n=30)		p
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	
Age, year	26.43	4.76	26.1	5.1	25.6	3.48	0.771
Gestational age, week	34.33	3.89	37.6	1.87	35.77	4.5	0.003**
Gravidity	1.7	1.02	1.93	0.94	1.73	1.11	0.638
Parity	0.4	0.62	0.63	0.76	0.5	0.86	0.49
Effacement	7	12.36	56.33	20.92	23.67	12.99	0.000**
Dilation	0.31	0.63	3.73	2.36	1.37	0.76	0.000**

n = total number, SS = Statistical Significance, SD = standard deviation. ** Significant at p<0.05.

The PAMG-1 test yielded statistically significantly higher positive results in the patients who presented with increased cervical dilation (Table 2).

Table 2. Distribution of patients according to PAMG-1 status

	PAMG-1 negative (n=43)		PAMG-1 positive (n=47)		p
	Mean	*SD*	*Mean*	*SD*	
Age, year	26.10	4.44	26.00	4.53	0.920
Gestational age, week	34.14	4.42	37.44	2.28	0.000**
Gravidity	1.71	1.13	1.85	0.92	0.520
Parity	0.48	0.80	0.54	0.71	0.683
Effacement	9.52	13.78	46.04	21.71	0.000**
Dilatation	0.43	0.63	3.00	2.10	0.000**

n = total number, SS = Statistical Significance, SD = standard deviation. ** Significant at p<0.05.

This difference was associated with positive PAMG-1 test results and higher cervical dilation rates in Group 2 who came to give birth and for whom labor started and PROM was detectable by conventional methods. Similarly, the PAMG-1 test also yielded statistically significant results for the cervical effacement. This effect was amplified by the PAMG-1-negative results in Group 1, who were naturally expected to be excluded from cervical effacement, as their pregnancies were uncomplicated, and as ROM was already ruled out by traditional methods. However, there was no significant difference between PAMG-1-positive and -negative results in terms of the gravidity, parity, the presence of vaginal infection, a history of coitus within the last 48 hours, or maternal age. The incidences of coitus, vaginal infection, and clinical chorioamnionitis of the patient groups are presented in Table 3.

Table 3. Incidence of coitus, vaginal infection, and clinical chorioamnionitis according to patient group.

		Group 1 (n=30)		Group 2 (n=30)		Group 3 (n=30)		X^2	p
		n	%	n	%	n	%		
Coitus	No	26	86.7	24	80.0	22	73.3	1.66	0.435
	Yes	4	13.3	6	20.0	8	26.7		
Vaginal infection	No	19	63.3	18	60.0	7	23.3	11.82	0.003**
	Yes	11	36.7	12	40.0	23	76.7		
Clinical chorioamnionitis	No	30	100	30	100	29	96.7	-	-
	Yes					1	3.3		

n = total number, **Significant at p< 0.05.

Table 4. Results of nitrazine, IGFBP-1, and PAMG-1 tests versus clinical diagnosis at the time of presentation

		ROM positive patients	ROM negative patients	Total
Nitrazine	positive	47	17	64
	negative	1	25	26
IGFBP-1	positive	44	2	46
	negative	4	40	44
PAMG-1	positive	47	0	47
	negative	1	42	43
Total		48	42	90

PAMG-1 = Placental alpha microglobulin-1, IGFBP-1 = Insulin-like growth factor binding protein-1, ROM = rupture of membranes.

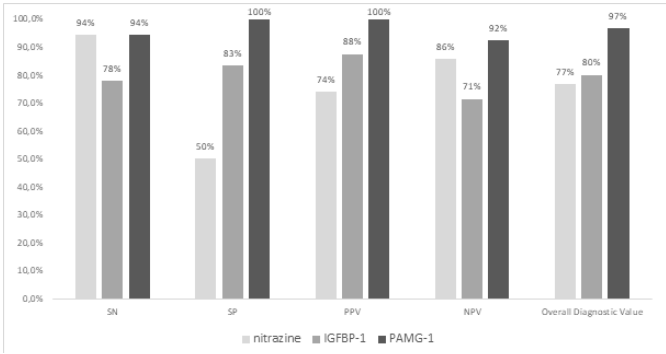
Table 5 shows the nitrazine, IGFBP-1, and PMAG-1 test results compared to the clinical diagnosis at the time of admission. Table 6 shows the metric performance of nitrazine, IGFBP-1, and PMAG-1 tests in all groups. The sensitivity, specificity, PPV, and NPV of nitrazine was 94.4%, 50.0%, 73.9%, and 85.7%, respectively. These values were 77.8%, 83.3%, 87.5%, and 71.4% for the IGFBP-1, respectively and 94.4%, 100%, 100%, and 92.3% for the PAMG-1, respectively. Accordingly, the PAMG-1 showed the highest diagnostic value in both the overall population and those with an equivocal status of ROM.

Table 5. Test performance for nitrazine, IGFBP-1, and PAMG-1 tests across all patient groups

Metric	NITRAZINE	IFGBP-1	PAMG-1
SN	97.9% (47/48)	91.7% (44/48)	97.9% (47/48)
(95% CI*)	(88.9%-99.9%)	(80.0%-97.7%)	(88.9%-99.9%)
SP	59.5% (25/42)	95.2% (40/42)	100% (42/42)
(95% CI*)	(43.3%-74.4%)	(83.9%-99.4%)	(91.6%-100%)
PPV	73.4% (47/64)	95.7% (44/46)	100% (47/47)
(95% CI*)	(60.9%-83.7%)	(85.2%-99.5%)	(92.5%-100%)
NPV	96.2% (25/26)	90.9% (40/44)	97.7% (42/43)
(95% CI*)	(80.4%-99.9%)	(73.7%-95.1%)	(87.7%-99.9%)
CDV	80%	93.3%	98.9%

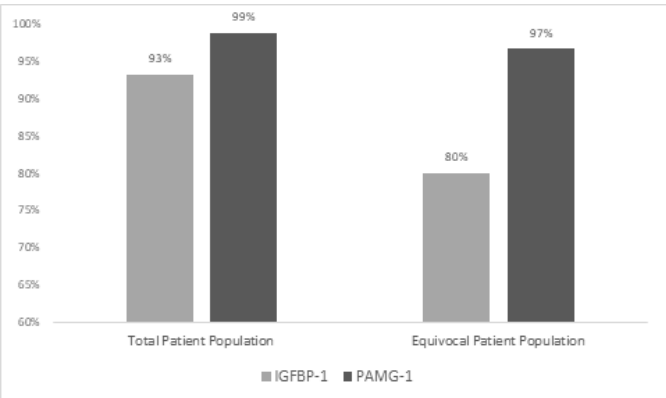
SN = sensitivity, SP = specificity, PPV = positive predictive value, NPV = negative predictive value, PAMG-1 = Placental alpha microglobulin-1, IGFBP-1 = Insulin-like growth factor binding protein-1, CDV, cumulative diagnostic value. *95% confidence interval (CI) computed by the Clopper-Pearson test.

Figure 1 shows the diagnostic performance of each test for the patients in Group 3.



SN = sensitivity, SP = specificity, 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.

Figure 2. IGFBP-1 and PAMG-1 test performance in cumulative diagnostic value in total patient population and equivocal patient population



PAMG-1 = Placental alpha microglobulin-1, IGFBP-1 = Insulin-like growth factor binding protein-1.

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