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Use of vaginal creatinine levels in detecting premature rupture of membranes

Erken memran rüptürü tespitinde vajinal kreatinin seviyelerinin kullanılması

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Abstract

Aim: Misdiagnosis is frequent in premature rupture of membranes (PROM) patients. The most accurate diagnosis of PROM requires reliable laboratory tests. Due to the lack of a gold-standard diagnostic method, many methods have been proposed in this regard. The aim of this study is to determine an easy diagnostic method in early membrane rupture and to determine the usability, reliability and cut-off values of vaginal creatinine measurements in the detection of PROM compared to vaginal placental alpha microglobulin-1 (PAMG-1) test.

Methods: We designed a cross-sectional study. A total of 63 patients admitted to the Obstetrics and Gynecology Clinic of Samsun Ondokuz Mayıs University with suspected PROM between 15 November 2012 and 15 June 2013 were included in this study. Following anamnesis, all patients were vaginally subjected to PAMG-1 (AmniSure® ROM) test with sterile speculum and injected with 5 cc of saline into the vagina, and then, a 3 cc sample was retrieved with the same injector and put into a biochemistry tube. Immediately thereafter, the material was sent to the biochemistry laboratory, centrifuged and stored at -70 ^{0}C until all samples were studied. The patients were classified as PROM and non-PROM based on the positive or negative result of PAMG-1 test. Following this classification, the patients were also grouped as PROM and non-PROM according to their vaginal creatinine values.

Results: The mean maternal age of our patients was 26.3 years in the PROM group and 28.8 years in the non-PROM group. The mean gestational weeks were 30.8 weeks in the PROM patients and 32.5 weeks in the non-PROM patients. Of 20 patients subjected to PAMG-1 test for PROM diagnosis, 17 were PAMG-1 and creatinine positive while 3 were PAMG-1 negative and creatinine positive. Of 43 patients subjected to PAMG-1 test, 42 were PAMG-1 negative while 1 was PAMG-1 positive and creatinine negative. Accordingly, vaginal creatinine was found to have 94.4% sensitivity, 93.3% specificity, 85% positive predictive value, and 97.7% negative predictive value in PROM diagnosis. The mean creatinine values in the PROM and non-PROM groups were 0.39 (0.31) mg/dl and 0.04 (0.10) mg/dl, respectively (P=0.05).

Conclusion: Creatinine assessment in vaginal flushing fluid can be a cheaper, faster, easily accessible and highly accurate test with 94.4% sensitivity and 93.3% specificity in PROM diagnosis.

Keywords: Creatinine, Premature rupture of membranes, Placental alpha microglobulin-1

Öz

Amaç: Erken membran rüptürü (EMR) bulunan hastaların teşhisinde yanılgılar sık görülebilmektedir. EMR tanısını en doğru şekilde koymak için güvenilir laboratuvar testleri gerekir. Altın standart bir tanı metodunun olmaması yüzünden bu konuda birçok yöntemler öne sürülmüştür Bu çalışmada EMR teşhisinde vajinal kreatinin ölçümlerinin %100 yakın güvenirlikle kullanılan vajinal Plasental Alfa mikroglobulin-1 (PAMG-1) testine göre kullanılabilirliği, güvenirliği ve cut off değerlerinin tespiti hedeflenmiştir

Yöntemler: Bu çalışmaya, Samsun Ondokuz Mayıs Üniversitesi Kadın Hastalıkları ve Doğum Kliniği'ne 15 Kasım 2012 – 15 Haziran 2013 tarihleri arasında başvuran toplam 63 EMR şüpheli hasta dahil edilmiştir. Tüm hastalara anamnezi takiben steril spekulum ile vajinal olarak PAMG-1 (AmniSure ® ROM) testi ve ardından vajene 5 cc serum fizyolojik enjekte edilip sonrasında 3 cc aynı enjektörle geri alınarak biyokimya tüpüne koyulmuştur. Hemen ardından materyal biyokimya laboratuvarına gönderilip santrifüj edildikten sonra dondurucuda -70 ^{0}C derecede tüm numuneler çalışılıncaya kadar saklanmıştır. Hastalar Plasental Alpha Mikroglobulin-1 (PAMG-1) testinin pozitif ve negatif olup olmamasına göre EMR olan ve EMR olamayan olarak sınıflandırıldı. Bu sınıflamanın ardından vajinal kreatinin değerine göre de hastalar EMR ve EMR olmayan olarak gruplandırıldı.

Bulgular: Olgularımızın ortalama maternal yaş değeri EMR olan hasta gurubunda 26,3 yaş, EMR olmayan gurupta 28,8 yaş idi. EMR olan hastalarda gebelik haftası ortalama değeri 30,8 hafta, EMR olmayan hastalarda ise 32,5 hafta idi. EMR tanısı amaçlı PAMG -1 testi uygulanan 20 hastanın 17 sinde PAMG-1 ve kreatinin pozitif, PAMG-1 negatif olan 3 hastada kreatinin pozitifti. PAMG-1 testi uygulanan 43 hastanın 42 sinde PAMG-1 negatif, 1 inde PAMG-1 pozitif ve kreatinin negatifti. Bu sonuçlara göre vajinal kreatinin EMR teşhisinde %94,4 sensitivite, %93,3 spesifisite, %85 pozitif prediktif değer, %97,7 negatif prediktif değer olarak hesaplandı. EMR olan ve olmayan gurupta ortalama kreatinin değerleri sırasıyla 0,39 (0,31)mg/dl ve 0,04 (0,10) mg/dl (P=0,05).

Sonuç: Vajinal yıkama sıvısında kreatinin ölçümü EMR tanısında daha ucuz, hızlı, kolay ulaşılabilir ve %94,4 sensitivite, %93,3 spesifisite ile yüksek doğruluk oranına sahip bir test olabilir.

Anahtar kelimeler: Kreatinin, Erken membran rüptürü, Plasental alfa mikroglobulin-1

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Introduction

Premature rupture of membranes (PROM) is defined as the rupture of fetal membranes before the onset of uterine contractions required for labor [1,2]. If PROM occurs before the 37th gestational week, it is called preterm PROM (PPROM). The PROM incidence is 5-10% of all births [3,4]. 60-80% of PROM occurs in term pregnancies and 20-40% in pregnant women before the 37th week. Although PPROM is observed in 3% of all pregnancies, it is responsible for or relevant to one third of preterm births. This rate further increases in multiple pregnancies [5]. PPROM is the most frequent cause of preterm births [6]. Despite the advances in medicine and technology, PROM and especially PPROM are still two troublesome issues causing therapeutic dilemmas in modern obstetrics, and they bring about several problems. These problems include prematurity, perinatal infections, oligohydramnios, umbilical cord compression, pulmonary immaturity, and the resulting risks associated with fetus such as increased perinatal mortality and morbidity. In addition, there are maternal risks such as increased cesarean section rate, choriodecidual infection, and placental decollement [7,8]. Misdiagnosis is frequent in PROM patients. While false positive results may lead to unnecessary interventions such as hospitalization or even the induction of labor, false negative results prevent or delay taking necessary obstetric measures such as the treatment of maternal infection [9,10].

The most accurate diagnosis of PROM requires a good history, physical examination and reliable laboratory tests. Due to the lack of a gold-standard diagnostic method, many methods have been proposed in this regard [4,11-15]. Previously, the combination of amniotic fluid pooling in the speculum examination, nitrazine test for pH determination, and fern tests based on microscopic evidence were used in the diagnosis of PROM. However, false positive results in these tests due to contamination with blood, semen and urine are substantial. Furthermore, there is gradually less diagnostic accuracy in the period following the rupture of membranes [16,17]. Such problems in diagnosis have paved the way for numerous tests that use biochemical markers. In search for a gold standard, alpha-fetoprotein (AFP), B-hCG, vaginal prolactin, fetal fibronectin, insulin-like growth factor binding protein-1, and placental alpha microglobulin-1 (PAMG-1) immunoassay tests have been the subject of many studies. In addition, although ultrasonography-guided intra-amniotic injection of indigo carmine is an effective test, its association with risk factors such as placental decollement, infection, iatrogenic PROM, and fetal loss has restricted its use. In recent years, PAMG-1 has come to the forefront among other non-invasive diagnostic methods in PROM diagnosis for reasons such as its high concentration in amniotic fluid and low concentration in the blood, and its low concentration in the cervicovaginal fluid while fetal membranes are intact. Although it is one of the most valuable diagnostic methods at the moment, its low popularity and expensiveness are regarded as its disadvantages.

Creatinine assessment in vaginal fluid has been the subject of several studies. Most of the amniotic fluid is formed by the transfer of fetal urine into the amniotic fluid as of the second half of the pregnancy. Urea, creatinine, and uric acid blend into amniotic fluid at high concentration through fetal urine. The mean creatinine value in amniotic fluid in early gestational period was found to be 0.6 mg/dl. This is equal to the creatinine value in the maternal blood [18]. The creatinine concentration in amniotic fluid increases very rapidly between 20th and 32nd weeks and reaches 2-4 times the value in the maternal blood [12]. When the threshold value of creatinine concentration in vaginal flushing fluid was accepted as 0.12 mg/dl in the study conducted by Gürbüz et al. [12], and the value of creatinine concentration was accepted as 0.6 mg/dl in the study conducted by Kafalı et al. [19], sensitivity, specificity, and positive and negative predictive values were found to be 100% in both studies. A study conducted by Zanjani et al. [20] found 96.7% sensitivity, 100% specificity, 100% positive predictive value and 96.8% negative predictive value when the threshold value of creatinine concentration was taken as 0.5 mg/dl. Moreover, creatinine value ranging between 1.5 and 2.0 mg/dl in the amniotic fluid was also observed as a symptom of fetal maturity [21]. In these studies, creatinine accuracy was identified in comparison to clinical assessment, nitrazine test and fern test. However, creatinine does not present the true diagnostic value due to the high false positive and negative rate of these conventional tests, and it has not been compared to tests such as PAMG-1 test with about 100% accuracy in the studies conducted on the use of vaginal creatinine levels in the PROM diagnosis..

Therefore, with this study, we aimed to compare PAMG-1 test, which is one of the recent diagnostic methods that can be considered as the gold standard but is expensive, less accessible, and with about 100% diagnostic accuracy, with creatinine in vaginal flushing fluid, which is more accessible, cheaper and with accuracy proven in recent studies.

Materials and methods

This article was approved by the Ethics Committee of the Faculty of Medicine of Ondokuz Mayıs University. Study type is "cross-sectional study". This study was carried out on 81 pregnant women admitted to Obstetrics and Gynecology Clinic at Samsun Ondokuz Mayıs University between 15 November 2012 and 15 June 2013. All the patients were given detailed information about the purpose of our study, the procedures to be followed and the estimated results of these procedures, and asked to sign the consent forms.

Women in the 20th-40th weeks of pregnancy who had water breaking complaint or referred with suspected water breaking were included in the study. 9 patients with vaginal bleeding, 3 patients with placenta previa and 6 patients lost to follow-up were excluded from the study. The study was carried out with a total of 63 patients.

Inclusion criteria

No history of preterm labor and premature rupture of membrane in previous pregnancies, pregnancy being in its second or third trimester, no fetal or placental pathology, no previously detected uterine pathology or malformation

Exclusion criteria

A significant amount of vaginal bleeding, presence of placenta previa, multiple pregnancies, no regular follow-up visits

All patients underwent vaginal examination with sterile speculum following the anamnesis. In vaginal examination, the

patients were evaluated for fluid pooling, fluid drainage from cervix during the Valsalva maneuver, cervical dilatation and effacement, cervicitis, vaginitis, blood, urine, meconium, and presence of semen. Next, the patients, who were admitted to our clinic with the complaint or pre-diagnosis of suspected water breaking, were subjected to the PAMG-1 test, which is implemented routinely in our clinic and has high diagnostic accuracy. Immediately afterwards, a vaginal flushing sample was taken for creatinine. Later, amniotic fluid indices (AFI) were assessed and recorded via ultrasonography with the four-quadrant technique. Demographics and obstetric characteristics of the patients such as maternal age, gravidity and parity were asked about and recorded in their first examinations.

Implementation of the PAMG-1 test

AmniSure ® ROM (Rupture of (fetal) Membranes Test) (International LLC, USA) test kit was used for PAMG-1 sampling. The kit includes one sterile polyester cotton swab, one tube containing sample resolution solution (0.5 ml), and one strip test in package. After the speculum was attached and the vagina and cervix were observed for sampling, the polyester tip of the cotton swab was inserted into the posterior vagina, holding the middle of the sterile polyester cotton swab and ensuring that it did not touch anywhere. A swab sample was also taken from the external cervical os surface and vaginal margins, and the cotton swab was removed from the vagina one minute later. With its polyester tip placed in the bottle containing the resolution solution, the cotton swab was rinsed by rotating for one minute. The cotton swab was then removed from the solution and disposed of, and the arrowed white tip of the test strip was plunged into the solvent vial for not less than five minutes and not more than ten minutes. If the test strip was clearly visible in the vial, it was removed after not less than five minutes, or once ten minutes were fully up. The test strip was placed on a clean, dry, flat surface, and the result was read and recorded. If only one control line was visible, the test result was considered negative; if both the control and test lines were visible, the test result was considered positive; and if no line was visible, the test result was considered invalid and the test was repeated.

Sampling for creatinine in vaginal fluid

Without removing the speculum after the PAMG-1 test, 5 cc saline was injected into the vagina, and then, 3 cc was retrieved into the biochemistry tube with the same injector. Immediately thereafter, the material was sent to the biochemistry laboratory, centrifuged and stored at -70 ^{0}C until all samples were studied.

The patients were classified as PROM and non-PROM based on the positive or negative result of PAMG-1 test. Following this classification, the patients were also grouped as PROM and non-PROM according to their vaginal creatinine values.

Statistical analysis

The data obtained from the study were coded, recorded and analyzed using SPSS (Statistical Package for Social Sciences) 15.0 software package. For data evaluation, continuous variables were expressed in mean (standard deviation) while the frequency data were expressed in numbers (%). Kruskal-Wallis, Mann Whitney U, N-Par and Chi-Square tests were used for statistical analysis. $P<0.05$ was accepted as the level for statistical significance.

Results

The gestational weeks of 63 patients who participated in our study were between 22 weeks and 36 weeks and 5 days. Following the PAMG-1 test, patients were classified as PROM and non-PROM. 18 patients were found to have PROM whereas PROM was not detected in 45 patients. PROM and non-PROM patients were demographically evaluated in the following tables. Table 1 shows the numerical distribution of the presence of PROM by gestational weeks according to being PAMG-1 positive or negative.

Table 1: Distribution of PROM by gestational weeks according to being PAMG-1 positive or negative

Gestational week	PROM	Non-PROM	Total	*P*-value
20- 27+6 weeks	4 (22.2%)	7 (15.6%)	11 (17.46%)	0.51
28-31+6 weeks	4 (22.2%)	8 (17.8%)	12 (19.04%)	0.52
32-35+6 weeks	8 (44.4%)	18 (40%)	26 (41.33%)	0.55
36-37 weeks	2 (11.1%)	12 (11.1%)	14 (22.22%)	0.58
TOTAL	18 (100%)	45 (100%)	63 (100%)	

No statistically significant difference was found between PROM and non-PROM patients who were admitted to our clinic by gestational weeks according to PAMG-1. 26 (41.33%) of the patients were pregnant for 32-36 weeks. In figure 1, presence of PROM is schematized by gestational weeks.

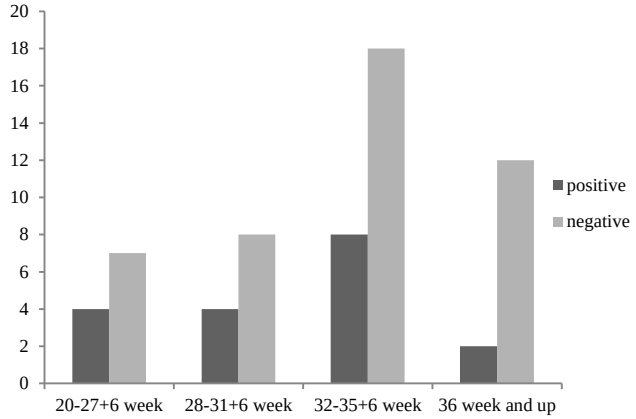

Figure 1: Numerical distribution of PROM by gestational weeks

The mean maternal age of our patients was 26.3 years in the PROM group and 28.8 years in the non-PROM group. The mean gestational weeks were 30.8 weeks in the PROM patients and 32.5 weeks in the non-PROM patients (Table 2).

Table 2: Comparison of mean maternal age and mean gestational weeks in terms of being PAMG-1 positive or negative

	PROM	Non-PROM	*P*-value
Maternal age Mean (SD), year)	26.38 (4.1)	28.08 (5.0)	0.23
Gestatıonal week Mean (SD), week)	30.84 (4.52)	32.54 (4.08)	0.24

No statistically significant difference was observed between the PROM and non-PROM patients by mean maternal age ($P=0.23$). There was no statistically significant difference between the PROM and non-PROM patients by mean gestational weeks ($P=0.24$). Mean cervical effacement of our patients was 34.4% in the PROM group and 16.6% in the non-PROM group. Mean cervical dilatation was 1.5 cm in the PROM group and 0.7 cm in the non-PROM group. Mean amniotic fluid index was 61.6 in the PROM group and 73.1 in the non-PROM group. Table 3 shows the mean values of cervical dilatation, cervical effacement and amniotic fluid index for PROM and non-PROM groups.

Table 3: Comparison of mean values of cervical dilatation, cervical effacement and amniotic fluid index in terms of being PAMG-1 positive or negative

	PROM	Non-PROM	*P*-value
Cervical effacement Mean (SD), %)	34.44 (18.22)	16.67(20.56)	0.01
Cervical dilatation Mean (SD), cm)	1.5 (0.70)	0.76 (0.95)	0.01
Amnion fluid index Mean (SD)	61.67 (26.40)	73.11(42.51)	0.48

Mean cervical dilatation and mean cervical effacement were significantly higher in the PROM group (P=0.01). This difference was attributed to the onset of labor in the PROM patient. This difference was attributed to the onset of labor in the PROM patient. There were no statistically significant differences between mean amniotic fluid indices (P=0.48). The reason was associated with the fact that the patients were referred to our clinic either for the complaint of water breaking or the suspicion of oligohydramnios-related water breaking. 22 of our patients were nulligravida. 7 of the nulligravida were in the PROM group and 15 in the non-PROM group. 41 patients were multigravida. 11 of the multigravida were in the PROM group and 30 in the non-PROM group (Table 4).

Table 4: Comparison of patients' gravidity status in terms of being PAMG-1 positive or negative

	PROM	Non-PROM	Total	*P*-value
Nulligravida	7 (38.88%)	15 (33.33%)	22 (34.92%)	0.92
Multigravida	11 (61.11%)	30 (66.66%)	41 (65.07%)	0.95

No significant difference was found in gravidity by the presence or absence of PROM in the patients (P=0.92 and P=0.95, respectively). As shown in figure 2, 38.8% of the patients suspected of PROM and accepted as having PROM were nulligravida, and 33.33% of the non-PROM patients were observed to be nulligravida.

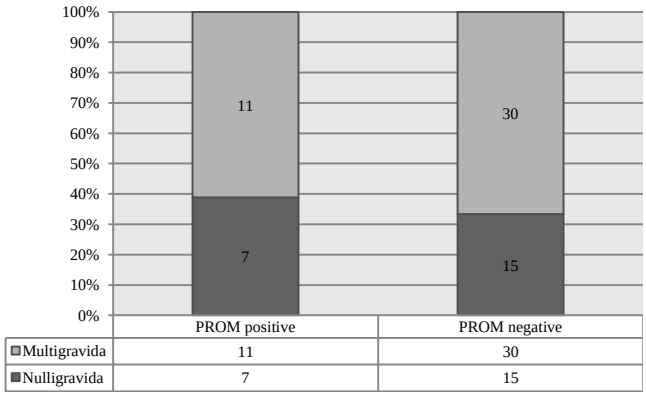

Figure 2: Gravidity distribution of the patients according to being PROM positive or negative

25 of our patients were nullipara. 8 of the nullipara were in the PROM group and 17 in the non-PROM group. 38 patients were multipara. 10 of the multipara were in the PROM group and 28 in the non-PROM group. The patients' parity status is compared according to the presence or absence of PROM in table 5.

Table 5: Comparison of patients' parity status in terms of being PAMG-1 positive or negative

	PROM	Non-PROM	Total	*P*-value
Nullipara	8 (44.44%)	17 (37.77%)	25 (39.68%)	0.55
Multipara	10 (55.55%)	28 (62.2%	38 (60.31%)	0.58

There was no significant difference between the parity statuses of the patients by the presence or absence of PROM. 44.4% of the patients suspected of PROM and accepted as having PROM were nullipara, and 37.7% of the non-PROM patients were observed to be multipara.

The mean time between sampling and delivery was 14.4 days in the PROM patients, and 27.4 days in the non-PROM patients. No statistically significant difference was found between these two groups. However, the median value of the time between sampling and delivery was observed to be 1 day in the PROM patients, and 21 days in the non-PROM patients. In table 6, the time between sampling and delivery was compared according to being PROM positive or negative.

Table 6: Comparison of the time between sampling and delivery according to the presence or absence of PROM

	PROM		Non-PROM	
Time between sampling and delivery (days)	Mean (SD)	Median (min-max)	Mean (SD)	Median (min-max)
	14.4 (31.6)	1 (0.25-112)	27.4 (22.37)	21 (0.25-106)

A statistically significant difference was observed between the vaginal flushing fluid creatinine levels of the PROM and non-PROM patients. In our study, mean creatinine level in vaginal flushing fluid was 0.39(0.31) mg/dl in the PROM group, and 0.04 (0.10) mg/dl in the non-PROM group (Table 7). Figure 3 shows the distribution of mean creatinine level in vaginal flushing fluid according to the presence or absence of PROM.

Table 7: Mean creatinine levels in the PROM and non-PROM groups

	PROM	Non-PROM	*P*-value
Creatinine mg/dl (SD)	0.39 (0.31)	0.04(0.10)	0.06

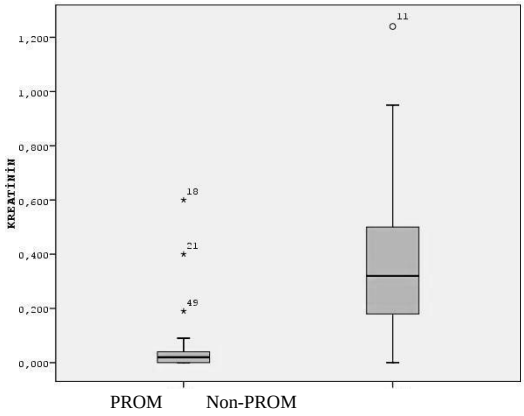

Figure 3: Distribution of mean creatinine levels in vaginal flushing fluid according to the presence or absence of PROM

Creatinine levels in vaginal flushing fluid in the patients classified as being PROM and non-PROM according to the PAMG-1 test were analyzed using ROC curve. According to the analysis results, when the creatinine cut-off value was taken as 0.1050 mg/dl, the patients with 0.1050 mg/dl or higher creatinine cut-off value were accepted as having PROM while the patients with creatinine cut-off value lower than 0.1050 mg/dl were accepted as having no PROM. Accordingly, 17 of 18 PAMG-1-positive patients were found to be vaginal flushing fluid creatinine positive. 42 of 45 PAMG-1-negative patients were found to be vaginal flushing fluid creatinine negative.

All things considered, when the cut-off value of creatinine in vagina flushing fluid was taken as 0.1050 at the end of the ROC curve analysis and compared with the PAMG-1 test, sensitivity, specificity, positive and predictive values were found to be 94.4%, 93.3%, 85%, and 97.7%, respectively (Table 8).

Table 8: Accuracy of creatinine in vaginal flushing fluid compared to PAMG-1 test

		PAMG-1		
		Positive	Negative	Total
Creatinine	Positive	17	3	20
	Negative	1	42	43
	Total	18	45	63

Sensitivity: 94.4%, Specificity: 93.3%, Positive predictive value: 85%, Negative predictive value: 97.7%, Total diagnostic value: 93.6%

Discussion

The incidence of PROM is 5-10% of all births [3,4]. Preterm PROM is observed in 3% of all pregnancies, but it is responsible for or associated with one third of preterm births [17]. Preterm PROM affects 120,000 pregnant women in the United States every year. It is associated with maternal, fetal, neonatal morbidity and mortality as it causes infection, umbilical cord compression, placental decollement, and prematurity. Therefore, a rapid and accurate diagnosis of PROM is very important. The majority of patients can be diagnosed with PROM upon observation of vaginal pooling and amniotic fluid leaking from cervix during speculum examination. However, when vaginal amnion leakage is not observed or when intermittent or minimal amnion leakage is observed, the diagnosis is suspicious. Bleeding, vaginal discharge, semen, and urine make the diagnosis difficult [17].

In 90% of the cases, PROM can be diagnosed through anamnesis, physical examination or conventional methods [17,8]. In cases where PROM cannot be diagnosed by conventional methods, the presence or absence of amniotic fluid in vaginal fluid should be determined quickly and reliably in order to confirm the PROM diagnosis. As an alternative to conventional methods such as nitrazine (pH) test, fern test, and pooling, which are commonly used in the diagnosis of PROM, the presence of proteins such as IGFB*P*-1, AFP, prolactin, fetal fibronectin and hCG, which are components of amniotic fluid, have been investigated in vaginal fluid. Since there is no test that can show rupture of membranes, especially cases of micro-rupture, at 100%, it is often not easy to make the right decision about the sensitivity and specificity of these tests [22]. PAMG-1 assay in vaginal fluid differs in this respect. There is no need for additional instrument or trained personnel to perform the test. As its diagnostic accuracy rate has been determined to be 99% in many studies, it has been approved and used as the most valuable diagnostic method today [4,23]. PAMG-1 was isolated in amniotic fluid by Petrunin et al. [24] in 1975 for the first time obtained the anti-PAMG-1 antibody and assessed the protein content and concentration in amniotic fluid at different stages of pregnancy with immunochemical methods.

Due to its high concentration in amniotic fluid, low concentration in blood, very low concentration in cervicovaginal fluid while fetal membranes are intact, and so on, PAMG-1 has been purposefully used as a diagnostic test in PROM cases. To minimize the false results in the test used in this study, two monoclonal antibodies that adjust the sensitivity level at the optimal low level were chosen. These low values were used to determine the amniotic fluid value of extremely low amounts in vaginal secretions (0.0025-0.00025 ml can determine the amount of amniotic fluid in 1 ml vaginal secretion). Background concentration of PAMG-1 using this combination of monoclonal antibodies is approximately 50- 220 picograms (0.05-0.22 ng) per 1 ml of vaginal fluid. The sensitivity cross section of the test is 5-7 ng/ml, which is at least 20 times higher than the ground-level concentration. It was ensured with this range to remove false negative and false positive results efficiently. In addition to the PAMG-1 test, another effective test is the IGFB*P*-1 (Insulin-like growth factor binding protein-1) test, which is more common in Europe than in the United States. This test has also high accuracy. In various studies, its sensitivity was found in the range of 93-98%, its specificity in the range of 95-100% and its positive predictive value about 98% [14,25,26]. However, in a meta-analysis study conducted in 2013, PAMG-1 test was found to be more accurate than IGFB*P*-1 test [27].

Based on these data, we routinely perform PAMG-1 test to confirm our diagnosis of patients suspected of having PROM in our clinic. We group the patients as having PROM and no PROM according to whether PAMG-1 test is positive or negative, and evaluate them accordingly. As the main theme of our study, we wanted to compare PAMG-1, through which we consider patients as positive or negative, with a cheaper, more accessible and applicable method with the same accuracy. In this regard, we thought that creatinine assessment in vaginal flushing fluid, which is proved by different studies in new publications, can be useful and effective. Previous studies on creatinine have found different cut-off values to diagnose PROM patients. We compared it with highly accurate PAMG-1 and started our study with the aim of determining both the most accurate cut-off value for the vaginal flushing fluid creatinine and the accuracy rate according to this cut-off value. The study included 63 patients. No statistically significant difference was found between the patients whom we considered having PROM or no PROM according to being PAMG-1 positive or negative in terms of mean maternal age, mean gestational weeks, and mean gravidity and parity. There was no statistically significant difference between the mean amniotic fluid indices of the groups. No significant difference in the mean amniotic fluid indices was associated with the fact that all the patients were referred to our clinic either for the complaint of water breaking or the suspicion of oligohydramnios-related water breaking and that the patients had similar characteristics. There was a statistically significant difference in mean values of cervical effacement and cervical dilatation in favor of the PROM patients. The mean values of cervical dilatation and effacement were higher in the PROM group than in the non-PROM group. The reason might be the fact that the patients in this group started preterm labor and that cervical dilatation and effacement could occur in patients with rupture of membranes. In our study, there was no statistically significant relationship between PROM and non-PROM group in terms of time between sampling and delivery. The mean time between sampling and delivery was 14 days and the median value was 1 day in the non-PROM group while the mean time was 27 days and the median value was 21 days in the PROM group.

When compared to the literature, we see that the study conducted by Dale et al. [28]. on 111 PPROM pregnant women between 20-34 weeks found the median value of the latent period to be 7 (0-109) days In our study, we thought that the mean was increased because the post-treatment latent period of two PROM patients were 112 and 80 days after the development of PROM. A statistically significant difference was observed between the vaginal flushing fluid creatinine levels of the PROM and non-PROM patients. In our study, mean creatinine level in vaginal flushing fluid was found to be 0.39 (0.31) mg/dl in the PROM group, and 0.04 (0.10) mg/dl in the non-PROM group. The study performed by Gürbüz et al. [12] also observed the same level to

be 0.026 (0.029) mg/dl in the group whose water did not break, and 0.70 (0.55) mg/dl in the group whose water broke. It has been suggested in previous studies that the creatinine assessment in vaginal flushing fluid can be used as a marker for diagnosis when PROM is clinically suspected in the speculum examination [12,16,19,20,30]. Firstly, the study conducted by Li Hy et al. [29] found that hCG, AFP and creatinine concentrations were high in amniotic fluid. They reported that the assessment of hCG, AFP and creatinine in vaginal flushing fluid was useful. They also stated that creatinine assessment in vaginal flushing fluid is cheaper and easier than hCG and AFP in PROM diagnosis. Secondly, Gürbüz et al. [12] compared the vaginal flushing fluid creatinine levels of 54 pregnant women in whom amnion flow was detected through speculum examinations with the creatinine levels of 34 pregnant women with no complaint. As a result, when the creatinine threshold was taken as 0.12 mg/dl, they observed sensitivity, specificity, positive and negative predictive values to be 100%. The third study on the subject was carried out by Kafalı et al. [19] from Turkey. They assessed the levels of urea and creatinine in vaginal flushing fluid in the PROM diagnosis, and found high levels of urea and creatinine in PROM patients. However, they studied only with the patients whose PROM diagnosis was confirmed with vaginal pooling and nitrazine test. They concluded that sensitivity, specificity, and positive and negative predictive values were all 100% when urea was taken as 12 mg/dl and creatinine as 0.6 mg/dl in vaginal flushing fluid. Based on this result, they argued that urea and creatinine assessment might be used as an easy, cheap and fast test in the PROM diagnosis.

Furthermore, in the study in which Zanjani et al. [20] confirmed PROM diagnosis in the speculum examination according to the presence of pooling and the nitrazine test result, they found 96.5% sensitivity, 100% specificity, 100% positive predictive value and 96.8% negative predictive value when the cut-off value of creatinine in the vaginal flushing fluid was taken as 0.5 mg/dl. However, the high false positivity rate of nitrazine test in the PROM diagnosis due to the possibility of change in vaginal pH in the presence of blood, semen or infection such as bacterial vaginosis has paved the way for other studies.. Sekhavat et al. [16] used fern test and the detection of pooling in the speculum examination to confirm the PROM diagnosis test on the grounds that nitrazine test has a high rate of false positivity. Accordingly, they found 98.7% sensitivity, 100% specificity, 100% positive predictive value and 98.8% negative predictive value when the creatinine cut-off value in the vaginal flushing fluid was taken as 0.14 mg/dl. In our study, the creatinine level in vaginal flushing fluid was calculated with ROC analysis by taking the cut-off value as 0.1050 mg/dl compared to PAMG-1, which is highly accurate in the PROM diagnosis. As a result, 94.4% sensitivity, 93.3% specificity, 85% positive predictive value, and 97.7% negative predictive value were found, and the total diagnostic value was observed to be 93.6%. Unlike other studies, we found a cut-off value of 0.1050 mg/dl for vaginal flushing fluid creatinine. This value was lower than those found in other studies. We attributed this to the higher accuracy of the PAMG-1 test with which we compared creatinine assessment in vaginal flushing fluid for the PROM diagnosis. Limitation of this study is that this research is a retrospective study.

Conclusion

PROM is one of the most troublesome issues in today's obstetrics as one of the most important causes of preterm births. The correct diagnosis of PROM is critical for both maternal and fetal concerns. While a false positive diagnosis leads to unnecessary hospitalization, a false negative diagnosis causes intrauterine infection, increasing morbidity and mortality of both mother and fetus. Therefore, many tests have been performed to confirm the PROM diagnosis. Today, PAMG-1 test is recognized as the most effective and valuable diagnostic method. While there have been attempts to develop several alternative tests, none of them has achieved such success. As shown in our study, we concluded that creatinine assessment in vaginal flushing fluid is a cheaper, faster, easily accessible and highly accurate test in the PROM diagnosis, and we think it might be an alternative to PAMG-1 test.

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