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Original Article

The measurement of vaginal fluid creatinine aids in the diagnosis of preterm membrane rupture

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Abstract

***Background & Aims*:** Pre-labour rupture of membranes is a critical obstetric condition, contributing significantly to maternal and neonatal morbidity and mortality. This study aims to investigate the diagnostic value of vaginal fluid creatinine in the identification of PROM.

***Materials & Methods*:** In this descriptive-analytical study, 150 pregnant women were recruited and randomly assigned to three groups: confirmed PROM (n = 50), suspected PROM (n = 50), and healthy controls (n = 50). Vaginal fluid samples were collected and analyzed for creatinine concentration using the Jaffe technique. Data were analyzed with SPSS version 16, employing ANOVA and post hoc tests.

***Results*:** The mean creatinine level in vaginal fluid was significantly higher in the PROM group compared with the control group ($p < 0.05$). The Tukey post hoc test showed significant creatinine level differences between PROM cases and controls. Maternal age, gestational age, and fetal weight did not show a significant correlation with creatinine levels ($p > 0.05$). Creatinine testing demonstrated higher diagnostic accuracy compared to the Fern test, with a moderate correlation rate of 54%.

***Conclusion*:** The measurement of creatinine in vaginal fluid is an inexpensive, quick, and dependable diagnostic test for PROM, which is superior to the Fern test. Vaginal creatinine testing may also enhance maternal and neonatal outcomes due to the timeliness and accuracy of early diagnosis of PROM.

***Keywords*:** Creatinine, Premature rupture of membrane, Vaginal discharge

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Introduction

PROM is defined as the rupture of fetal membranes before the onset of labour and can occur at any time in pregnancy, either at or above term pregnancies of ≥ 37 weeks, or before less than 37 weeks in preterm pregnancies (1). PROM is considered to be a significant clinical event since it is associated with maternal and neonatal complications such as infection, preterm delivery, and increased rates of morbidity and mortality (2).

PROM affects approximately 3–8% of all pregnancies and accounts for 30–40% of preterm births,

which are the leading cause of neonatal deaths and long-term complications (3). Studies indicate that PROM contributes to approximately 18–20% of fetal deaths and up to 21% of neonatal complications (3). Term PROM occurs more frequently than preterm PROM; however, the latter is associated with greater risks due to the immaturity of the fetus and the higher likelihood of complications such as respiratory distress syndrome (RDS) (3).

The complications associated with PROM are multifaceted. For the mother, PROM can result in intrauterine infections such as chorioamnionitis (10–20%) and postpartum endometritis, both of which increase the risk of sepsis and other severe outcomes (3). For the fetus, PROM significantly raises the likelihood of preterm birth, umbilical cord prolapse, and hypoxia-related complications such as fetal asphyxia (3-4). In cases of PROM, the primary contributors to neonatal mortality include infection (10–15%), hypoplasia, and limb deformities (4).

The exact etiology of PROM is not fully understood, but several risk factors have been identified (6). These include infections of the cervix or vagina, previous history of PROM, smoking, low socioeconomic status, nutritional deficiencies, and uterine over distension caused by polyhydramnios or multiple gestations (6). Structural abnormalities of the fetus, short cervical length (< 2.5 cm), and early pregnancy bleeding are also associated with increased PROM risk (5-6). Given the potential for severe outcomes, prompt and accurate diagnosis of PROM is critical for guiding appropriate clinical management (3). Traditionally, PROM has been diagnosed using a combination of clinical assessments and diagnostic tests. Physical examination, including the observation of amniotic fluid leakage during sterile speculum examination, is often the first step. However, this approach is subjective and can yield inaccurate results if leakage is minimal or absent at the time of examination (6).

PROM can be confirmed using many biochemical tests, including the Fern test and nitrazine test (6). In the Fern test, dried vaginal fluid is examined microscopically; if it contains amniotic fluid, the fluid crystallizes with a characteristic ferning pattern after drying (6). The nitrazine test relies on changes in pH as amniotic fluid is more alkaline compared to normal vaginal secretions. These simple tests are prone to false positives and negatives, both (3-6). Contaminations with semen, cervical mucus, or urine are a few of the reasons for false positives, while the false negatives can be seen in prolonged intervals since membrane rupture due to the absorption or dilution of amniotic fluid (6). The nitrazine test relies on the usual alkaline pH of the cervicovaginal secretions. It is the most commonly used test on a daily routine for the diagnosis of PROM (7).

Although this test is burdened with a high false positive rate, it is the most frequently performed test in the diagnosis of PROM. The fern test carries a high possibility of being both false positive and false negative. It is dependent on the microscopic crystallization of amniotic fluid upon drying, which is often collected using a sterile swab from the posterior fornix of the vagina. The false negatives are due to mainly technical error or contamination with blood, while false positives are due to contamination with cervical mucus or semen and fingerprints. Sensitivity and specificity of the fern test were reported as 51% and 70%, respectively, in patients without labour and 98% and 88%, respectively, in patients in labour (8). Other tests are usually used only when conventional tests for PROM do not give a confirmatory diagnosis. These include the amnio-dye test. On the other hand, the invasive ones like the amnio dye test will give a definite confirmation with risks of infection, induction of preterm labour, or even placental abruption. (3, 9) These limitations define the necessity for non-invasive, correct, and cost-effective diagnostic alternatives. Recent advancements in PROM diagnosis have focused on identifying biochemical markers in vaginal fluid. These markers include human chorionic gonadotropin (HCG), alpha-fetoprotein, prolactin, fetal fibronectin, urea, and creatinine (10-15). Of these, urea and creatinine have emerged as promising diagnostic tools due to their relatively higher concentrations in amniotic fluid compared to vaginal secretions (16). These markers are particularly appealing because they are non-

invasive, inexpensive, and easy to measure using standard laboratory techniques. The rationale for assessing these markers is their elevated concentration in AF relative to typical vaginal discharge. These tests rely on diagnosing vaginal lavage using one or more biochemical markers present in the PROM environment, which are absent in women with intact membranes. However, despite the promising diagnostic potential of these markers, their complexity and high cost have prevented their widespread acceptance (17).

Creatinine, a byproduct of muscle metabolism, is primarily excreted through the kidneys (18). During pregnancy, the fetus begins producing urine around 8–11 weeks of gestation, contributing significantly to the amniotic fluid composition in later stages of pregnancy (18). Consequently, creatinine concentrations in amniotic fluid are markedly higher than in vaginal secretions (19). Measuring creatinine levels in vaginal fluid provides a very good indication of amniotic fluid leakage and has proved to be highly sensitive and specific in several studies (20-21). Other studies also highlighted the cost-effective and practical nature of the test for creatinine, especially in resource-poor settings where facilities for advanced diagnostic equipment might not exist (20-21).

This study will appraise the diagnostic value of creatinine levels of vaginal fluid in diagnosing PROM. This study will compare the creatinine testing method against the traditional methods, Fern tests, to ensure its accuracy and cost-effectiveness as a diagnostic tool. The findings are expected to contribute to the standardization of protocols that would undergird creatinine testing and improve the clinical outcomes for pregnant women with PROM.

Materials & Methods

One hundred fifty pregnant women with a singleton pregnancy and a gestational age of 28–40 weeks, admitted to Shahid Motahari Hospital of UMSU, were enrolled in this observational study. The Ethics Committee of Urmia University of Medical Sciences authorized this article (IR.UMSU.REC.1398.263). The study group comprised 100 pregnant women reporting vaginal fluid leakage (case group) and 50 normal pregnant women, selected randomly, who attended the prenatal clinic for routine check-ups without any complaints or difficulties (control group). The sonographic assessment during the first trimester of pregnancy established the gestational age. All women in the initial group underwent a sterile speculum examination to verify the presence of AF emanating from the cervix. The “confirmed PROM group” (group I) received positive results, while the “suspected PROM group” (group II) received reports of vaginal fluid leakage without obvious AF flow or pooling and a negative fern test each group comprised 50 participants. All women received sonographic evaluations to ascertain AF and gestational age. Upon acquiring written consent from patients, we documented their demographic information and collected samples. In instances of flowing/pooling AF, creatinine sampling involved aspirating vaginal fluid after injecting 3 mL of sterile water into the posterior vaginal fornix using the same syringe. The control group initially injected 5 mL of sterile water into the posterior vaginal fornix, then aspirated 3 mL using the same syringe. We promptly dispatched the samples to the laboratory, centrifuged them, and stored them at -30 °C. Women were excluded due to fetal anomalies, intrauterine fetal demise, known medical conditions, prenatal complications, visible blood in vaginal secretions, and use of vaginal medications or intercourse the previous night, meconium in AF, multiple gestations, and regular uterine contractions. We quantified vaginal creatinine using the Jaffe technique. The Jaffe reaction, a colorimetric assay, measures the levels of creatinine in urine and blood. The creatinine in the sample reacts with the reagent to quantitatively generate an orange hue with picric acid in an alkaline environment. After a 15-minute incubation at room temperature, we measured the color at 520 nm. The color alteration is directly proportional to the concentration of creatinine. The Jaffe reaction, while being an antiquated, nonspecific test for creatinine, remains the preferred method for creatinine testing due to its rapidity, adaptability in automated analysis, and cost-effectiveness. The secretions were

initially centrifuged, after which 500 µl of the supernatant was extracted for further analysis. We then introduced the liquid into a calibrated spectrophotometer and used light absorption measurements to determine the creatinine concentration. We gathered and analyzed all demographic and clinical information pertaining to each patient using SPSS software version 16.

Results

A total of 150 pregnant women were included, with a mean age of 27.8 ± 6 years, ranging from a minimum of 15 to a maximum of 39 years. In the frequency distribution study, the duration of pregnancy was 10% under 34 weeks and 90% above 34 weeks (Table 1). The mean fetal weight was 682 ± 2,753 g, with a minimum weight of 1100 g and a maximum weight of 4200 g. The mean creatinine level in vaginal discharge was 0.15386 ± 0.2158 with the lowest creatinine level being 0.01 and the highest being 0.7. In the study of the frequency distribution of the number of previous pregnancies, 34% had no previous pregnancies, 19.3% had one previous pregnancy, and 29.3% had two previous pregnancies. The maximum number of previous pregnancies was six. The frequency distribution of the Fern test results 24.7% positive and 75.3% negative.

Table 1. Demographic characteristics of pregnant women referred to Urmia Shahid Motahari Hospital.

		Percentage	**Abundance**
Duration of pregnancy	Under 34 weeks Over 34 weeks	10% 90%	15 135
Number of previous pregnancies	0 1 2 3 4 5 6	34% 19.3% 29.3% 13.3% 2.7% 0.7% 0.7%	51 29 44 20 4 1 1
Fern test	Positive Negative	24.7% 75.3%	37 113
Total		100	150

There was no significant relationship between gestational age and creatinine levels in the vaginal discharge of pregnant women (p value > 0.05). There was no significant relationship between maternal age and creatinine levels in the vaginal discharge of pregnant women (p value > 0.05). The ANOVA test showed that there was a significant difference in creatinine levels in the vaginal discharge of pregnant women with AF leakage compared to healthy pregnant women and women suspected of amniotic sac rupture (p value < 0.05) (Table 2). The Tukey test showed that creatinine levels in the vaginal secretions of healthy pregnant women in one group and creatinine levels in the vaginal secretions of pregnant women with AF leakage and women suspected of amniotic sac rupture in the other group were significantly heterogeneous (p value < 0.05. Therefore, the amount of creatinine in the vaginal discharge of pregnant women with AF leakage and those suspected of amniotic sac rupture was higher than the amount of creatinine in the vaginal discharge of healthy pregnant women. (0.1104). There was no significant relationship between fetal weight and creatinine levels in the vaginal discharge of pregnant women (p value > 0.05). There was no significant relationship between the number of previous pregnancies and the amount of creatinine in the vaginal discharge of pregnant women (p value > 0.05). There was a significant relationship between the accuracy of the vaginal fluid creatinine test and Fern test (p value < 0.05). The Cramer's correlation coefficient between the

accuracy of vaginal fluid creatinine test and the Fern test was 54%, indicating a moderate relationship (Cramer's V = 54%, P < 0.05) (Table 3). Of the 50 cases of amniotic sac rupture, 28 (56%) were correctly diagnosed by the Fern test, while44% were not correctly diagnosed. Of the 50 cases of ruptured amniotic sacs, the Fern test was 100% accurate. Of the 50 suspected amniotic sac ruptures, the Fern test was positive in 18% of cases and negative in 82% (Table 4).

Table 2. Comparison of creatinine in vaginal discharge of pregnant women with amniotic fluid leakage, healthy pregnant women, and women suspected of amniotic sac rupture using ANOVA

	Total Square	Abundance	Mean Square	F	Significant Level
Between groups	0.361	2	0.181	4.564	0.012
Intragroup	5.820	147	0.04		
Total	6.181	149			

Table 3. Accuracy comparison between Fern and creatinine tests

Suspicious		Normal		Rupture	
Cr	Fern	Cr	Fern	Cr	Fern
28% Cr top of cut off	18% positive	18% Cr top of cut off	0% positive	56% Cr top of cut off	56% positive

Tabel 4. Statistics on creatinine in the vaginal discharge of pregnant women referred to Urmia Shahid Motahari Hospital.

The amount of creatinine in vaginal discharge of pregnant women (Suspicious)	The amount of creatinine in vaginal discharge of pregnant women (control group)	The amount of creatinine in vaginal discharge of pregnant women (PROM)
50	50	50
0.2132 ± 0.2791	0.1104 ± 0.1311	0.2158 ± 0.15386
0.1	0.07	0.1950
0.02	0.01	0.01
1.1	0.72	0.7

Discussion

Our study found no significant correlation between fetal weight and the number of prior pregnancies in the vaginal secretions of pregnant women. A substantial correlation existed between the accuracy of the vaginal fluid creatinine test and the Fern test. The Cramer's correlation coefficient between the accuracy of the vaginal fluid creatinine test and the Fern test was 54%, indicating a moderate link. Multiple studies on the identification of PROM using vaginal creatinine levels show that this method is straightforward, cost-effective, and reliable for diagnosing PROM (22, 23). The findings of this research align with those of the current study. These studies determined that creatinine measurement is a viable and straightforward diagnostic method, particularly as it is far less expensive than hospitalization or alternative tests. Tiwari et al. (24) achieved the most effective diagnosis of PROM among the four diagnostic biomarkers—beta HCG, prolactin, creatinine, and alpha-fetoprotein. The findings of this investigation align with our research. In their investigation of the concentrations of urea, creatinine, and B-HCG in vaginal fluid for diagnosing PROM, Ghasemi et al. (13) demonstrated a significant elevation in all four levels in the case group, with B-HCG and prolactin demonstrating superior diagnostic value. Their study's findings differed from ours because we only measured creatinine in our investigation, and their study's variables did not match ours. Li HY et al. (20) discovered that assessing creatinine in vaginal fluid was more cost-effective and simpler than measuring hCG. In a separate investigation, Gurbuz et al. (21) indicated that assessing vaginal fluid creatinine with a threshold of 0.12 mg/dL and achieving sensitivity, specificity, negative predictive value (NPV), and 100% positive predictive value (PPV) in the diagnosis of premature membrane rupture was more cost-effective and expedient than alternative methods. Kafali et al. demonstrated that measuring urea or creatinine in vaginal fluid for the detection of PROM is a reliable, straightforward, and rapid test, achieving 100% sensitivity, specificity, NPV, and PPV with cut-off values of 12 and 0.6 mg/dL, respectively. They hypothesized that vaginal creatinine readings would serve as a diagnostic tool for fetal development in instances of preterm delivery, as creatinine levels in AF are contingent upon gestational age. Using urea and creatinine in vaginal fluid to check for PROM is a simple, reliable, and quick test that has high sensitivity, specificity, PPV, NPV, and a fair amount of accuracy. This method is ideal for frequent use as it requires no additional equipment, is universally accessible, and is cost-effective for PROM; it serves particularly well as an auxiliary test in difficult PROM situations. Different sample sizes, inclusion criteria, and the gestational age of the examined subjects may account for variations in cut-off levels among studies. To make the results of this study even more solid, more research should be done with larger sample sizes and more diverse populations. This research should also compare the accuracy of the vaginal creatinine test with other ways to diagnose PROM, such as AF and fetal fibronectin testing.

Conclusion

PROM is a significant concern in contemporary obstetrics, serving as a primary contributor to preterm births. The accurate identification of PROM is essential for both maternal and fetal considerations. The findings of this study indicated a moderate correlation between the vaginal fluid creatinine test and the Fern test; nevertheless, the accuracy of the vaginal fluid creatinine test is superior to that of the Fern test. Our work has shown that creatinine evaluation in vaginal flushing fluid is a cost-effective, rapid, readily accessible, and highly precise method for diagnosing PROM

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Author's Contributions

Tahereh Behroozi-lak1, Sonia Sadeghpour designed the study. Neda Hamdolahpour Collected data. Tahereh Behroozi-lak1, Sonia Sadeghpour, Neda Hamdolahpour analyzed the data. All of the authors revised and approved the paper.

Data Availability

The data supporting these findings are available from the corresponding author upon reasonable request.

Conflict of Interest

Authors declare there is no conflict of interest.

Ethical Statement

This study was approved by the Ethics Committee of Urmia University of Medical Sciences (code IR.UMSU.REC.1398.263) in accordance with ethical principles.

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