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Vaginal fluid creatinine in diagnosing pre-labor rupture of membranes in Vietnam

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Abstract

Introduction: Pre-labor rupture of membranes (PROM) is a common issue in obstetrics that can lead to serious complications. Timely diagnosis is crucial for proper management of PROM. Creatinine concentration in vaginal fluid is a potential candidate for diagnosing PROM in resource constrained environment. This study aims to investigate the usability of creatinine concentration and its optimal cutoff value for diagnosing PROM.

Methods: We conducted a case-control diagnostic test study at Hung Vuong Maternity Hospital, Vietnam. Pregnant women between 24–42 weeks were enrolled into case and control groups, with a ratio of 1:2. We used sterile speculum examination for fluid leak from cervix, nitrazine test and ferning test are used to determine membrane's status. Vaginal fluid was collected by injecting sterile saline and later withdrawing. Data was summarized using descriptive statistics, difference between groups was tested using Mann Whitney U test. The cutoff value was determined by receiver operating characteristics (ROC) curve and Youden's J statistic. Ethics approval was obtained from the local ethics committee, all participants gave written informed consent. We recruited a total of 693 pregnant women into the study – 231 participants in the study group and 462 participants in the control group.

Results: The median vaginal fluid creatinine concentration in the study group is significantly higher than those in the control group (0.84 mg/dL versus 0.09 mg/dL, p<0.01). The optimal cutoff for diagnosing PROM was 0.29 mg/dL, providing 93% sensitivity, 97% specificity and 95% accuracy.

Conclusions: Vaginal fluid creatinine concentration is a credible indicator for PROM, providing great diagnostic power and high-quality information.

Keywords: fetal membranes, premature rupture (PROM: pregnancy); ROC curve; amniotic fluid; creatinine

1. INTRODUCTION

Pre-labor rupture of membranes (PROM) happens in 10 percent of normal pregnancy, of which 2 to 4 percent occur in preterm pregnancy. PROM can cause several serious consequences, including infection and high risk of preterm birth [1],[2]. Despite its importance, there are nearly 30 percent of cases are underdiagnosed or over diagnosed [3],[4]. Timely and accurate diagnosis of PROM is crucial in clinical practice. On one hand, overdiagnosis can lead to unnecessary

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intervention, affecting both maternal and fetal morbidity and mortality. These includes unnecessary hospitalization, inappropriate antibiotic usages or termination of pregnancy. On the other hand, underdiagnosis can delay necessary interventions, such as antibiotic, administration of steroids, or timely termination of pregnancy. This can increase the risk of umbilical cord prolapse, fetal distress, chorioamnionitis and placental abruption.

Many laboratory tests have been developed to diagnose ruptured membranes. Most authors agreed that the gold standard for non-invasive test in clinical practice is the combination of vaginal discharge with the following criteria: (1) amniotic fluid leak from cervix or pooling in posterior vaginal wall via speculum examination, (2) a positive Nitrazine test and (3) a positive ferning test. There are many cases where one of these criteria is not met, which restrict us from a definitive diagnosis [5],[6]. This is a challenge in clinical practice. To overcome this issue, many methods have been proposed, including injecting colored chemical indicator into the amniotic fluid and observing the leaking fluid. However, this is an invasive method, which has serious adverse effects on both mother and the child. Therefore, it is almost no longer used in clinical practice [7].

Recent advances in technology have introduced many novel tests to diagnose PROM. These tests were based on the detection of certain components of amniotic fluid in the vaginal fluid, including: fetal fibronectin, measuring vaginal pH level, alpha-fetoprotein, insulin growth factor binding protein-1, human chorionic gonadotropin, prolactin [8]–[10]. However, their low sensitivity and specificity combined with high prices prevented the usage of these tests in clinical practice. Recently, FDA had approved a minimally invasive rapid diagnostic test for ruptured membranes – PMAG-1 (AmniSure PROM test), with high sensitivity and specificity (98%–99%, respectively). Despite its accuracy, the price of this test is not suitable for lowmiddle-income countries (LMICs). It remains a challenge to develop a reliable, simple to use and affordable test that can be widely applied in resource-constrained healthcare facilities.

Quantitative test for creatinine in vaginal fluid is a potential candidate to address this need. The test is based on the assumption that most of the amniotic fluid produced in the second and third trimester is the fetus urine. Therefore, normal compounds in urine, namely creatinine, will have a much higher concentration in the amniotic fluid compared to the vaginal fluid. Detecting a high concentration of creatinine in vaginal fluid will be a reliable indicator for ruptured membranes. It is reasonable to try using creatinine to determine the membrane status. Recent studies had tested this hypothesis, the results showed that creatinine in vaginal fluid can help to diagnose ruptured membranes with high sensitivity and specificity [11]–[13]. However, there have not had many studies to prove this, and they tend to have a small sample size. The cutoff values are inconsistent between studies, which can be difficult for doctors to use these in clinical practice. There is an unmet need for larger study to investigate the use of creatinine in diagnosing PROM. This study aimed to assess the usability of creatinine in a LMICs settings and identify the appropriate cutoff value for creatinine concentration in vaginal fluid.

2. METERIALS AND METHODS

2.1. Study settings

This study was conducted at Hung Vuong Maternity Hospital, Ho Chi Minh City, Vietnam. Recruitment was conducted from the June 15th, 2021 to the March 1st, 2022.

2.2. Study design and participants

2.2.1. Study design

This was a case-control diagnostic test study. Pregnant women were enrolled into 2 groups: one group with confirmed membranes ruptured (case group) and the other group with membrane intact (control group). The ratio between the case group and control group is 1:2.

2.2.2. Study participants

Pregnant women at the gestational age between 24–42 weeks were selected for this study. Women who visited the hospital antenatal clinic and women who presented to the emergency room for suspected membrane rupture without active contractions were eligible for the study. One member

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of the study team will screen patients for their eligibility, with the exclusion criteria as follows: age younger than 18 years-old, could not provide informed consent, a history of renal failure, recent vaginal bleeding, still birth, or the fetal has renal tract abnormalities.

2.2.3. Data collection and tools

Upon obtaining the informed consent, all study participants were asked about their history of illness, signs of suspected membrane rupture, general and abdominal examination. Sterile speculum examination was performed to check for presence of fluid flowing from the cervix and free fluid in posterior fornix. Nitrazine and ferning tests were carried out by a member of the study team. If the nitrazine paper changed its color from yellow to blue, it was being considered as positive. Thereafter, vaginal washing fluid was collected for creatinine measurement as follows: doctors injected 5 mL of sterile saline into the posterior vaginal fornix, waited for 30 seconds, and used the same syringe to withdraw approximately 3 mL of fluid. The sample was then sent to the laboratory department for creatinine concentration measurement within 30 minutes. We used Jaffe chemical calorimetric method to determine the concentrations of creatinine in vaginal fluid samples.

We used a convenient sampling method for the case group. PROM was diagnosed where all three following conditions are met: (1) a positive fluid leak from cervix or free fluid in posterior fornix during sterile speculum examination; (2) a positive nitrazine test;and (3) a positive ferning test. Pregnant women presented in the emergency department with a history of vaginal fluid leakage and diagnosis confirmed by the above-mentioned criteria will be included in the case group. The control group recruited women with matching gestational age from the antenatal clinic and must have a negative fluid leak, a negative nitrazine test and a negative ferning test. Participants who did not meet these criteria will be excluded from the analysis and their vaginal fluid samples will be disposed.

After completing the study procedure, patients continued with hospital routine protocol for managing diagnosed PROM pregnant women (case group) or normal clinic visit (control group). Information related to demographic and obstetric characteristics, examination reports and test results are all documented in the case report form.

2.3. Sample size and sampling

Sample size calculation was based on the estimation of area under the curve (AUC) in diagnosis of PROM by creatinine concentration in vaginal fluid. From previous study [14] we chose the AUC=0.902, with α=0.05, estimation error=0.035 and ratio between case to control is 1:2. Based on these value, the calculated sample size were 215 for the case group and 430 for the control group.

2.4. Statistical method

Data entry was performed using Microsoft Excel 2013 (Microsoft®, Redmond, WA, USA), the principal investigators are responsible for validation after first data entry. Data cleaning and analysis were performed using RStudio version 1.4.1103 (Rstudio, Boston, MA, USA, PBC). Categorical variables were presented as number and ratio. Continuous variables were presented as mean, SD for normally distributed variable and as median, interquartile range for non-normal distribution. The Shapiro-Wilk test was used for normality testing. Numerical variables were compared between case group and control group using independent student's t-test or Mann Whitney U test, depending on variable's distribution. Diagnostic accuracy was assessed using the following index: sensitivity, specificity, and accuracy. Receiver operating characteristic (ROC) curve and Youden Index J = Max (Sen + Spe−1) were used to identify an optimal cutoff value. A p-value less than 0.05 was considered significant.

2.5. Ethical considerations

The study had received approval from the local ethics committee. All pregnant women who participated in the study had been consulted and provided written informed consent.

3. RESULTS

From June 2021 to March 2023, we have screened 729

potential pregnant women and recruited 693 participants. In the case group, we screened 248 pregnant women, of which 5 women had at least 1 exclusion criteria and 12 women did not have all 3 criteria to confirm PROM. In the control group, we screened 477 pregnant women, of which 11 women had at least 1 exclusion criteria and 4 women had at least one criterion for diagnosing ruptured membrane. Therefore, we recruited 231 participants in the case group and 462 participants in the control group, all are included in the analysis. Demographic data for each study group is summarized in Table 1.

All vaginal fluid samples were of good quality, and we were able to measure the concentration of creatinine in these samples. The creatinine concentration variable is not normally distributed. The median of creatinine concentration in the case group (0.84 mg/dL interquartile range (IQR): 0.53–1.22 mg/dL) is higher than that of the control group (0.09 mg/dL IQR: 0.05–0.16). There was a significant difference between the two groups in term of creatinine concentration ($p<0.01$).

ROC curve analysis and Youden's J statistic were used to determine the optimal cutoff point for creatinine concentration in vaginal fluid. The chosen cutoff value was 0.29 mg/dL, proving the highest Youden's J statistic with 95% accuracy, 93 sensitivity and 97% specificity (Fig. 1).

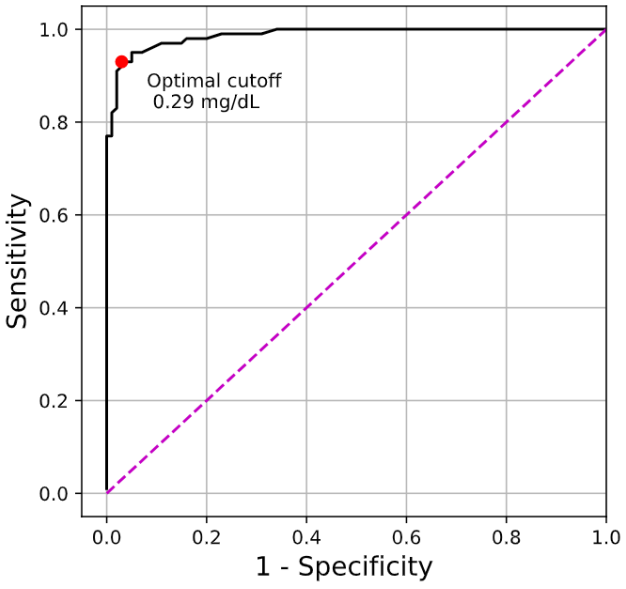

Fig. 1. Receiving operator characteristic curve for vaginal fluid creatinine.

4. DISCUSSION

Our study showed that the concentration of creatinine in vaginal fluid can be a good indicator for PROM. The most appropriate cutoff point for this test as a diagnostic of PROM was >0.29 mg/dL, which gives a sensitivity of 93% (95% CI: 89%–95%) and a specificity of 97% (95% CI: 94%–98%).

Table 1. Demographic characteristics of groups

Characteristics	Case group (n=231)	Control group (n=462)	p-value
Maternal age (years)	29 [25–33]	29 [27–33]	0.046
Gestational age (weeks)	38 [35–38]	38 [35–38]	0.5
Gravida	1 [0–1]	1 [0–2]	0.03
Parity	0 [0–1]	1 [0–1]	0.006

p-value<0.05 significant.
Independent students t-test.

Timely and accurate diagnosis plays a crucial role in the management of PROM. This has led many scientists to investigate different types of tests to help identify and confirm the membrane status. In resource limited settings, availability and cost-effectiveness methods are important factors, which explains why vaginal creatinine concentration has been studied widely. The following is a brief summary of key studies in this field.

Gurbuz et al. in 2004 [12] were among the first to investigate the value of creatinine concentration of vaginal fluid in PROM. The case-control study included 54 pregnant women at third trimester who were diagnosed with PROM by a positive pooling test, and 34 pregnant with intact membrane. The mean gestational age of participants was 36 weeks. Exclusion criteria were fetal defect affects the production of urine, vaginal bleeding, and placenta previa. The procedure included inserting 3 mL saline and withdrawing the fluid, the sample was tested using creatinine kit test Ektachem Clinical Chemistry Slides, Johnson & Johnson. The mean creatinine was 0.026±0.029 mg/dL in the control group and 0.7±0.55 mg/dL in the case group ($p<0,001$). The cutoff value was 0.12 mg/dL, provided 100% sensitivity and specificity. The study suggested that creatinine could be a candidate for a standard PROM test. However, the small sample size and imbalance

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between case and control group required deeper investigation.

In 2007, Kafali et al. conducted a case-control study in Turkey, recruited 47 pregnant women in the case group and 56 pregnant women in the control group [13]. There was a statistical difference in gestation age between two groups, with 35.5 weeks in case group and 40.1 in the control. Membrane rupture was defined as a positive pooling test and a positive nitrazine test. The mean creatinine concentration was 1.5±0.3 mg/dL in the case group and 0.28±0.23 mg/dL in the control group. Cutoff point was chosen at 0.6 mg/dL, which gave 100% for both sensitivity and specificity. These value are higher than other studies, which might be affected by race, ethnicity, nutrition and cultural as pointed out by Bouzani [15].

In 2013, Kariman et al. compared the value of vaginal creatinine and vaginal urea for diagnosing PROM [16]. The study recruited 179 pregnant women between 14 and 41 weeks of gestation, in Taleghani Hospital, Iran, of which, 126 women had complaints about vaginal fluid leakage and 53 women without any complaints as the control group. The study concluded that creatinine had a higher diagnostic power, providing 100% sensitivity and specificity at the cut-off value of 0.45 mg/dL.

The study conducted by Mohamed et al. in 2011 also found a high diagnostic power of vaginal creatinine, provided 100% sensitivity and specificity with the cut-off value of 0.31 mg/dL [17]. Begum et al. also found a similar cut-off value – 0.3 mg/dL in a study conducted in India. This cut-off value gave 90% sensitivity and 93.83% specificity [1].

In 2020, Malchi et at conducted a systematic review to investigate the accuracy of vaginal creatinine concentrations in the detection of PROM [18]. They included 11 studies with a total of 1,324 patients. The results indicated that the mean of creatinine in the case group was significantly higher than those in the control group. The test estimated sensitivity was 0.98 (95% CI: 0.92–0.99) and the specificity was 0.97 (95% CI: 0.89–0.99). However, the authors noted that the heterogeneity between studies was high and confidence interval (CI) of diagnostic odds ratio was high – suggesting a need for bigger studies to confirm the results.

Our study had several strengths. We followed clear criteria for diagnosing PROM. It included examinations and well-known laboratory tests. The study procedures were clearly defined, simple to follow and implemented without disrupting routine protocol at our local hospital. Due to constraints in time and resources, the study has its limitations. Case-control diagnostic test design could introduce bias, and the study sample might not fully represent the whole population. The convenient sampling method restricted us from determining the prevalence of PROM at our local hospital. This prevented us from identifying the positive predictive value and negative predictive value, which are highly valuable in clinical application.

5. CONCLUSION

Creatinine concentration in vaginal fluid has the potential to become a diagnostic test for PROM, especially in resource-limiting settings. The advantages of the test are non-invasive, high sensitivity, specificity combines with low cost and simple to implement in standard hospital procedure. This test can provide valuable information for decision-making, from normal patients to controversial cases. The differences in cutoff value from other studies might be attributed by differences in sample size and measurement methods. Future studies could investigate the development of a rapid test to detect creatinine concentration in vaginal fluid, providing a simple, accurate and convenient method to diagnose PROM.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics Approval

Ethics approval was obtained from the local ethics committee, all participants gave written informed consent.

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