Arch Gynecol Obstet (2012) 286:25–28
DOI 10.1007/s00404-012-2233-6

MATERNAL-FETAL MEDICINE

Practicability of vaginal washing fluid creatinine level in detecting premature rupture of membranes

Leila Sekhavat · Raziah Dehghani Firouzabadi · Prisa Mojiri

Received: 3 September 2011 / Accepted: 16 January 2012 / Published online: 25 January 2012

Abstract
Objective The purpose of this study was to evaluate the reliability of vaginal washing fluid creatinine level for the diagnosis of premature rupture of membranes (PROM).
Method A prospective diagnostic study performed in Shahid Sedoughi Hospital on 160 pregnant women (30 definite PROM, 30 no PROM and 100 suspected PROM) at 28–40 weeks of gestation. The vagina was washed by injection with a syringe filled with 3 ml of saline solution, and the washing fluid was collected from the posterior vaginal fornix and send to laboratory. Creatinine values in vaginal washing were measured and compared.
Result The mean vaginal fluid creatinine levels in definite PROM group, suspected PROM and no PROM were 0.40 ± 0.20, 0.16 ± 0.04 and 0.08 ± 0.01 mg/dl, respectively, where the difference was statistically significant ($P = 0.001$). The sensitivity, specificity, positive and negative predictivity values and accuracy were 98.7, 100, 100, 98.8 and 87.1%, respectively, in detecting PROM by evaluation of vaginal fluid creatinine concentration with cut-off value of 0.14 mg/dl.
Conclusion This study showed that creatinine determination in vaginal washing fluid is a useful marker for PROM diagnosis. It is a reliable, simple, cheap and rapid test.

L. Sekhavat (✉) · P. Mojiri
Shahid Sadoughi University of Medical Sciences, Yazd, Iran
e-mail: sekhavat@ssu.ac.ir; lsekhavat@yahoo.com

R. D. Firouzabadi
Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Keywords Premature rupture of membrane (PROM) · Vaginal washing fluid · Creatinine

Introduction

Premature rupture of membranes (PROM) is defined as the rupture of chorioamniotic membranes prior to the onset of labor and occurs in 10% of all gestations and about 2–4% of preterm pregnancies, with complications such as infection and preterm birth [1–3]. PROM is the cause of approximately one-third of preterm deliveries. It increases the risk of prematurity and leads to a number of other perinatal and neonatal complications, including a 1–2% risk of fetal death [4].

The diagnosis of PROM is sometimes challenging. A false positive diagnosis of PROM may lead to inappropriate intervention and a false negative diagnosis of PROM may cause maternal morbidities [5]. Diagnosis of PROM is easy when the rupture is obvious but difficult and indeed impossible when the rupture is minimal. The diagnosis of PROM requires a thorough history, physical examination, and selected laboratory studies [4]. For decades, a combination of visual pooling of amniotic fluid during speculum examination, alkaline pH determination and microscopic evidence of ferning has been widely used to determine rupture of membranes. Furthermore; these tests are prone to false positive results secondary to vaginal contamination with blood, urine, or semen [6]. To reduce false positive rates, several biochemical markers in the vaginal fluids have been studied, including alpha fetoprotein (AFP), human chorionic gonadotropin (hCG), prolactin, and fibronectin; and alpha microglobulins have been used in clinical studies to diagnose PROM [4, 7–10]. Such tests are based primarily on the identification in the cervicovaginal discharge of one or more biochemical markers that are present in the setting

of ROM, but absent in women with intact membranes. All these tests have advantages and drawbacks. Up to now there is no gold standard diagnostic test for PROM [9].

In the second half of gestation, most of the amniotic fluid comes from fetal micturition and fetal urine contributes significantly to the formation of amniotic fluid in the third trimester. Urea, creatinine, and uric acid are fetal waste products that are excreted in high concentration in fetal urine. It has been reported that pregnant women in the early gestational age has a mean creatinine concentration of 0.6 mg/dl in the amniotic fluid, similar to that found in maternal serum [2]. Creatinine concentration in amniotic fluid increases gradually between 20 and 32 weeks of gestation and more rapidly thereafter when they were two to four times higher than maternal serum [11].

We hypothesized that vaginal fluid creatinine may be helpful in diagnosing PROM because fetal urine is the most important source of amniotic fluid in the second half of pregnancy. Based on this hypothesis the current study was performed to evaluate the reliability of vaginal washing fluid creatinine level for the diagnosis of PROM.

Methods

This paper is the result of obstetrics and gynecology speciality thesis. A prospective diagnostic study performed in Shahid Sedoughi Hospital in Yazd, with the complaint of vaginal fluid leakage at 28–40 weeks of gestation from May 2009 to October 2010. The adopted protocol was approved by University Ethical Committee. All women were interviewed individually by the researcher. Written informed consent was obtained from all the patients.

Sample size estimations were based on the results of a previous study, and assuming a level of $\alpha = 0.05$ and $\beta = 0.6$ and difference 0.3 between definite PROM and no PROM, 160 patients were needed (30 definite PROM, 30 no PROM and 100 suspected PROM).

The women were excluded if there was the presence of any amount of vaginal bleeding (either spontaneous or traumatic due to the speculum examination), infective vaginal discharge, any prenatal complication (like preeclapsia, diabetes or UTI) and multiple pregnancies.

At admission, amniotic index measured by ultrasonography for all women. Then all patients underwent a clinical examination with sterile speculum. Amniotic fluid pooling with or without valsalva maneuver was noted. The ferning test was applied for all. PROM patients had documented confirmatory tests, including visualized pooling of amniotic fluid in the vaginal exam that was confirmed by nitrazine-positive result and demonstrated ferning. Patients who were pooling (+) and ferning (+) were taken as "definite PROM group". Patients who were pooling (±) and/or ferning (±) were taken as "suspected PROM group". Patients who were pooling (−) and ferning (−) were taken as "no PROM group". All the speculum examinations and ultrasonographic examinations were done by the same physician.

The vagina was washed by injection with a syringe filled with 5 ml of saline solution, and then 3 ml washing fluid was collected from the posterior vaginal fornix. The collected fluid was promptly quantitatively tested for the presence of creatinine with a creatinine assay (Ektachem Clinical Chemistry Slides, Johnson & Johnson) with a threshold of 25 mIU/mL. All tests were done by the same lab and by the same person.

Then all the patients were followed up until delivery and gestational age at delivery time. The parameters (age, parity, gestational age at delivery, time interval between sampling and delivery, vaginal fluid urea and creatinine) were compared.

Data were analyzed by SPSS with the T test, χ^2, Anova and Mc Nemar. Roc curve analysis was used to establish an optimal cut-off concentration. A P value <0.05 was considered statistically significant.

Results

Demographic data for each group are represented in Table 1. There were no significant differences in maternal age, gravidity and parity between groups.

Table 2 shows that gestational age at delivery and time interval between sampling and delivery was significantly shorter in definite PROM group than suspected PROM and no PROM group (35.1 ± 3.8, 38.3 ± 2.1 and 38.5 ± 2.3, respectively, $P = 0.03$).

Amniotic fluid index in definite PROM group was significantly lower than suspected PROM and no PROM group (62.7 ± 18.3, 83.3 ± 27.3 and 98 ± 14.5, respectively, $P = 0.001$). The mean vaginal fluid creatinine levels in definite PROM group, suspected PROM and no PROM were 0.40 ± 0.20, 0.16 ± 0.04 and 0.08 ± 0.01, respectively, where the difference was statistically significant ($P = 0.001$) (Table 2).

Receiver operating characteristic (ROC) curve analysis was used to establish the optimal cut-off concentration for vaginal washing fluid creatinine levels and it is found that cut-off value of 0.14 mg/dl is optimal (Fig. 1). The sensitivity, specificity, positive predictably, and negative predictably were 98.7, 100, 100 and 98.8, respectively, in detecting PROM by evaluation of vaginal fluid creatinine concentration with this cut-off value.

Discussion

PROM is an important obstetric problem, the failure of diagnosis of which can lead to unwanted infectious

Table 1 Demographic data of the groups

	Definite PROM ($n = 30$)	Suspected PROM ($n = 100$)	No PROM ($n = 30$)	P value
Maternal age (years) (mean ± SD)	26.5 ± 5.1	27.2 ± 4.7	28.0 ± 5.0	0.06
Gravidity, N (%)				0.3
Nuligravid	11 (36.7)	50 (50)	12 (40)	
Multigravid	19 (63.3)	50 (50)	18 (60)	
Parity				0.1
Nullipara	12 (40)	56 (56)	13 (43.3)	
Multipara	18 (60)	44 (44)	17 (66.7	
Gestational age (weeks) (mean ± SD)	35.1 ± 3.8	38.3 ± 2.1	38.5 ± 2.3	0.03
Interval between sampling and delivery (weeks) (mean ± SD)	1.4 ± 1.9	4.2 ± 2.1	6.3 ± 3.8	0.001

Table 2 Clinical characteristics and vaginal creatinine levels of groups

	Definite PROM	Suspected PROM ($n = 100$)	No PROM ($n = 30$)	P value
Gestational age (weeks) (mean ± SD)	35.1 ± 3.8	38.3 ± 2.1	38.5 ± 2.3	0.03
Interval between sampling and delivery (weeks) (mean ± SD)	1.4 ± 1.9	4.2 ± 2.1	6.3 ± 3.8	0.001
Amniotic fluid index (mean ± SD)	62.7 ± 18.9	83.3 ± 27.3	98 ± 14.5	0.001
Vaginal fluid creatinine (mg/dl) (mean ± SD)	0.40 ± 0.20	0.16 ± 0.04	0.08 ± 0.01	0.001

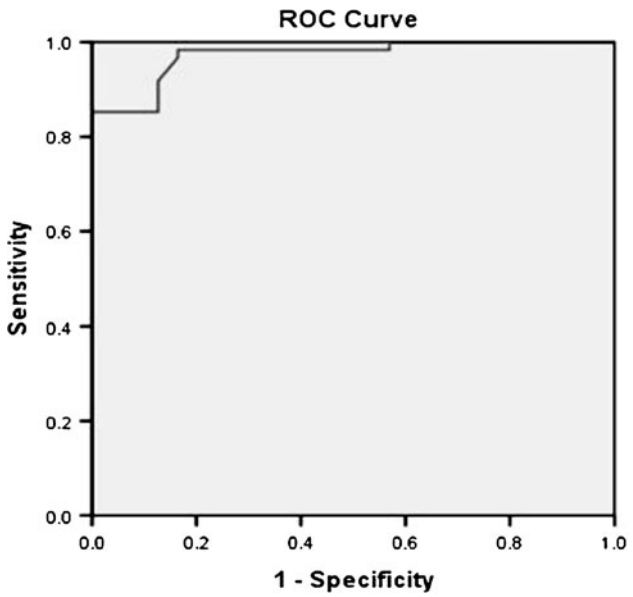

Fig. 1 The ROC curve for creatinine levels in vaginal washing

morbidity like chorioamnionitis and imminent term or preterm labor; on the other hand overdiagnosis can lead to unnecessary interventions like hospitalization. Therefore, its correct diagnosis has great importance. The approach to the diagnosis of membrane rupture is clinical, but clinical complaint of the patient is not reliable. Therefore, the current study was performed to evaluate the reliability of vaginal washing fluid creatinine level for the diagnosis of PROM. In our study, 130 pregnant women at 28–40 weeks of gestation with the complaint of vaginal fluid leakage presence or a suspicious history of it and 30 pregnant women in the same gestational age with no history of vaginal fluid leakage as control group were investigated. Results showed that the mean vaginal fluid creatinine levels in definite PROM group were higher than suspected PROM and no PROM groups.

Vaginal fluid creatinine determination has been suggested as a marker for PROM when the diagnosis remains in doubt after initial speculum examination by recent studies [9, 11, 12]. Li Hy et al. found that the concentrations of hCG, AFP and creatinine are high in amniotic fluid. They determined the usefulness of vaginal fluid hCG, AFP and creatinine measurements in the detection of PROM and found that creatinine was less expensive and easier to measure than hCG and AFP, and appears to be more accurate than hCG [9]. The second study was from Turkey. In this study Kafali et al. [12] used urea and creatinine determination to diagnose PROM. They reported

"Vaginal washing fluid urea and creatinine determination for the diagnosis of PROM is a reliable, simple and rapid test". In their study both urea and creatinine were high in confirmed PROM. Criterions for confirmed PROM were pooling (+) inspeculum examination and nitrazine (+). The nitrazine test can be "falsely positive" if the vaginal pH is increased by blood or semen contamination or alkaline antiseptics, or if bacterial vaginosis is present. Our study used positive ferning test and pooling for confirming PROM. The third study was accomplished by Gurbuz et al. at 2004. Gurbuz et al. [11] reported that the sensitivity, specificity, positive predictivity, and negative predictivity were all 100% in detecting PROM by evaluation of vaginal fluid creatinine concentration with a cut-off value of 0.12 mg/dl. In our study, the sensitivity, specificity, positive predictably, and negative predictably were 98.7, 100, 100 and 98.8, respectively, in detecting PROM by evaluation of vaginal fluid creatinine concentration with cut-off value of 0.14 mg/dl. In the study of Gurbuz et al. there were only two groups: women with the diagnosis of PROM that established only by inspection of vaginal pooling and the control group consisted of women with intact membranes. Our study consist suspected PROM that criterions for confirmed PROM were pooling (±) inspeculum examination and ferning test (±).

Our data show that vaginal fluid creatinine is an extremely useful marker in doubtful cases of PROM. In these cases, new methods such as AFP, beta-hCG, prolactin, insulin like growth factor binding protein 1 (IGFBP-1) and fetal fibronectin were investigated [7, 9, 13–15]. However, they have low specificity owing to overlap between the values of AFP, hCG, and fibronectin in patients with and without intact membranes. In our study, we were able to demonstrate an excellent PPV with reasonable sensitivity by creatinine measurements on cervicovaginal washings in a sample of patients with the status of the membrane intactness known. These findings are promising as this simple and inexpensive test may be helpful in determining the presence of PPROM in equivocal cases.

Therefore, we recommend vaginal washing fluid creatinine measurement for the diagnosis of PROM because of the following reasons. It has high specificity and reasonable sensitivity. It has a reasonable cost. Result of the test can be taken rapidly. This test does not be affected by presence of any amount of vaginal bleeding.

However, study should be continued with more cases for obtaining better results and transferring into practical work.

Conclusion

Our study shows that the creatinine measurement of cervicovaginal washings is a nonexpensive and fast method, and has higher sensitivity and specificity to establish accurate and valid diagnosis of PROM. It is a possible candidate to be a gold standard test.

Acknowledgments We would like to thank Mrs Naghshin and residents of obstetric and gynecologist of Shahid Sadoughi University of Medical Sciences for helping in gathering of data. We also thank Mr Zare for helping in data analysis.

Conflict of interest Authors do not have any conflict of interest.

References

1. El-Messidi A, Cameron A (2010) Diagnosis of premature rupture of membranes: inspiration from the past and insights for the future. J Obstet Gynaecol Can 32(6):561–569
2. Waters TP, Mercer B (2011) Preterm PROM: prediction, prevention, principles. Clin Obstet Gynecol 54(2):307–312
3. Simhan HN, Canavan TP (2005) Preterm premature rupture of membranes: diagnosis, evaluation and management strategies. BJOG 112(1):32–37
4. Medina T, Hill A (2006) Preterm premature rupture of membranes: diagnosis and management. Am Fam Physician 73(4):659–664
5. Kim YH, Park YW, Kwon HS, Kwon JY, Kim BL (2005) Vaginal fluid beta-human chorionic gonadotropin level in the diagnosis of premature rupture of membranes. Acta Obstet Gynecol Scand 84:802–805
6. Friedman ML, McElin TW (1969) Diagnosis of ruptured fetal membranes, clinical study and review of the literature. Am J Obstet Gynecol 104(4):544–550
7. Esim E, Turan C, Unal O, Dansuk R, Cengizoglu B (2003) Diagnosis of premature rupture of membranes by identification of beta-HCG in vaginal washing fluid. Eur J Obstet Gynecol Reprod Biol 107:37–40
8. Ni CY, Jia WX, Yi WM, Feng LH, Yu LZ (2003) Practicability of using vaginal fluid markers in detecting premature rupture of membranes. Ann Clin Biochem 40(5):542–545
9. Li HY, Chang TS (2000) Vaginal fluid creatinine, human chorionic gonadotropin and alpha-fetoprotein levels for detecting premature rupture of membranes. Zhonghua Yi Xue Za Zhi (Taipei) 63(9):686–690
10. Birkenmaier A, Ries JJ, Jens Kuhle J, Bürki N, Lapaire O, et al (2012) Placental α-microglobulin-1 to detect uncertain rupture of membranes in a European cohort of pregnancies. Arch Gynecol Obstet 285(1):21–25
11. Gurbuz A, Karateke A, Kabaca C (2004) Vaginal fluid creatinine in premature rupture of membranes. Int J Gynecol Obstet 85:270–271
12. Kafali H, Cevdet Öksüzle C (2007) Vaginal fluid urea and creatinine in diagnosis of premature rupture of membranes. Arch Gynecol Obstet 275(3):157–160
13. Buyukbayrak EE, Turan C, Unal O, Dansuk R, Cengizoğlu B (2004) Diagnostic power of the vaginal washing-fluid prolactin assay as an alternative method for the diagnosis of premature rupture of membranes. J Matern Fetal Neonatal Med 15(2):120–125
14. Erdemoglu E, Mungan T (2004) Significance of detecting insulin-like growth factor binding protein-1 in cervicovaginal secretions: comparison with nitrazine test and amniotic fluid volume assessment. Acta Obstet Gynecol Scand 83(7):622–626
15. Caughey AB, Robinson JN, Norwitz ER (2008) Contemporary diagnosis and management of preterm premature rupture of membranes. Rev Obstet Gynecol 1(1):11–22