The diagnostic value of the lactate level in the vaginal fluid for determining the premature rupture of membranes

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Abstract

Aim: To determine the diagnostic value of the lactate concentration in vaginal secretions in determining the premature rupture of membranes (PROM) in pregnant women at the Alavi Educational Treatment Center in Ardebil 2010.

Methods: This diagnostic clinical trial enrolled 100 pregnant women with a single pregnancy at the gestational age of 20–41 weeks. The women were divided to two groups of 50; the case group enrolled women with the premature rupture of membranes, and the control group consisted of women with intact membranes. To verify the PROM in both groups, the speculum, fern, and nitrazine tests were used. A Lactate Pro manual instrument was used to measure the lactate level of the vaginal fluid via enzymatic staining. A 5-µm aliquot of vaginal fluid was sufficient to determine the lactate level, which was displayed on the instrument's liquid crystal display after 60 seconds. Descriptive analytic statistics and SPSS 17 software were used to analyze the data.

Results: The threshold of the lactate concentration was determined to be 4.6 mM. A sensitivity of 96%, specificity of 98.8%, accuracy of 97%, positive predictive value of 97.95%, and negative predictive value of 96.07% were determined for the lactate concentration in the vaginal fluid for the diagnosis of the PROM.

Conclusion: Testing of the lactate level of the vaginal fluid appears to be an easy, rapid and valid method with high specificity and sensitivity that can be applied for the diagnosis of the PROM in pregnant women.

Key words: lactate level, vaginal fluid, rupture, membranes.

Introduction

Introduction: The premature rupture of membranes (PROM) is defined as the spontaneous rupture of embryonic membranes before labor contractions at any gestational age (1,2,3) and occurs in 2–25% of pregnancies. Its major complications are infections in the woman and unborn child, umbilical cord prolapse, prenatal mortality, and premature labor.(4, 5) PROM accounts for 30% of instances of premature labor (6, 7) and 18–20% of prenatal mortality. (8) Therefore, its accurate diagnosis is of great importance because a false positive diagnosis can lead to antibiotic therapy, corticosteroid therapy, or even labor induction. (9) However, the inability to diagnose PROM can lead to complications such as chorioamnionitis and preterm labor. (8, 10) The diagnosis of PROM is varied and usually based on clinical evaluations such as observing the fluid discharge from the cervix during speculum testing, observing the fern model in microscopic tests, and biochemical tests. (11) Among the biochemical tests, the detection of nitrazine, vaginal di-amine oxydase, prolactin, alpha fetoprotein, insulin-like growth factorbinding protein-1, human chorionic gonadotropin, fibronectin, and amniSure placental alpha macroglobulin-1 are frequently used. (12, 13) Most of these tests have

low sensitivity and specificity, have high negative and false positive rates, or are invasive. (14) Diagnosis based on clinical findings fails in 10% of cases. (15) Furthermore, the speculum test has a false negative rate of12%.(16) The nitrazine test is 90–97% sensitive and 16–70% specific, with a false negative rate of 9.4% and a false positive rate of 17.4%. The false positive readings arise from vaginitis, cervicitis, or contamination with blood, semen, urine, meconium, or antibiotics. The fern test is 51% sensitive and 70% specific. False negative readings can result from an incorrect sampling technique or contamination with blood or vaginal fluid, and the false negative rate ranges from 12.9 to 48.6%. False positive readings can be caused by contamination with cervical mucus or semen as well as an incorrect sampling technique, and the false positive rate ranges from 5.8 to 30%. (8, 10, 5) Recently, a new method has been devised for the diagnosis of PROM that relies of the measurement of lactate levels in the vaginal fluids. This method has gained popularity because it is easy to perform, can be performed at the patient's bedside, does not require advanced equipment, is relatively inexpensive, and is highly reliable and valid. Lactate is a metabolite generated from anaerobic metabolism and is in indicator of tissue hypoxia. Lactate is mostly produced in the myometer and/or chorio deciduas and is transferred to the amniotic fluid through membranes. (4, 16, 17) High concentrations of lactate (7–9 mM/l) can be detected in amniotic fluid (16); the amount of lactate in the amniotic fluid is 4–6 times higher than that in the fetus's or mother's blood (16, 18). Iberg-Itzel et al (2005) tested the diagnostic value of the lactate level in the vaginal fluids for the determination of PROM and estimated a sensitivity of 86% and a specificity of 92%. The positive predictive value was 92% and the negative predictive value was 87%. The timely and accurate diagnosis of PROM is critical. The measurement of the lactate concentration in the vaginal fluids is an easy, inexpensive, rapid, non-invasive, and available diagnostic method. Because few studies have addressed this technique, the present study was conducted to determine the diagnostic value of the lactate concentration in vaginal fluids for the determination of PROM in pregnant women at the Alavi Educational Therapeutic Center .

Methods

This diagnostic clinical trial enrolled 100 pregnant women who attended the pregnancy care clinic and midwifery emergency room of Alavi Educational Therapeutic Center in Ardebil. A data form was used to collect data in this study including demographic data, midwifery information, conditions of sample selection, manual Lactate Pro data, test tape for Lactate Pro data, nitrazine tape data, slide data, microscopy data, the observation checklist for recording the results of the examination using the speculum test, nitrazine test data, fern test data, and the lactate level of the vaginal fluids. Periodic calibration and standard control tapes were used to validate the enzymatic staining method. The expiration date of Japanese-made tapes (Okra, Japan) was also checked. The reputation of the manufacturing company (Okra, Kyoto, Japan) was used to validate the Lactate Pro equipment. The information form and observation check list were validated with regard to content validity. The test re-test method was used to check the reliability of the information form. Questions with a correlation over 0.85 were accepted. To confirm the reliability of the nitrazine and fern tests, 5 samples were taken from a pregnant woman and their correlation was evaluated (correlation coefficient=0.97).To confirm the reliability of the nitrazine test, fern test, and testing for the lactate concentration of the vaginal fluid, the inter observer method (kappa coefficient =0.86) was used. Participants were selected using convenient sampling. Based on the prevalence of PROM, α=0.05, and β=0.20 were applied to estimate a sample size of 50 in each group. Women who were pregnant with one fetus at a gestational age of 20–41 weeks with the chief complaint of leakage and who attended the pregnancy care clinic and midwifery emergency room of the Alavi Educational Therapeutic Center in Ardebil entered the study. Exclusion criteria included known fetal abnormality, known fetal asphyxia, fetal death, known diseases, known pregnancy complications, visible bloody vaginal fluid, application of vaginal medication the night before, intercourse the night before, meconium in the amniotic fluid, and regular contractions. To determine the gestational age, the participants had to know the exact date of their

last menstrual period, have had a sonogram before week 14, or have had two harmonious sonograms between 14 and 24 weeks. After written informed consent was obtained, pregnant women (PROM and control groups) who met the inclusion criteria enrolled in the study. The women filled out the preliminary questionnaire and took the NST test. The participants were then placed in the lithotomy position for speculum testing to observe fluid leakage from the cervix. Based on the speculum test results, participants were divided to two groups of positive and negative. The observations were recorded in the checklist. Immediately after the speculum test, the nitrazine test was performed. A swab was used to obtain a sample from the posterior fornix. The swab was then drawn on a strip of nitrazine paper. The color was read against the colors and numbers on the nitrazine package. A pH higher than 6.5 was considered to represent the rupture of the membranes. The results of the nitrazine test were recorded as positive or negative in the check list. A swab was then used in the same way to obtain a sample of vaginal fluid, which was drawn on a slide. After drying, the slide was examined with a light microscope at 10x magnification. Observation of the fern model was considered to represent a positive fern test. The results were recorded as positive or negative in the check list. To determine the amount of lactate in the vaginal fluid, a sample of vaginal fluid was collected on the posterior arm of the speculum. The sample was transferred to a strip of paper that was already coated with the enzyme and attached to the Lactate Pro equipment. The amount of lactate in the vaginal fluid was studied using enzymatic staining by Lactate Pro equipment. An aliquot of only 5ml of vaginal fluid was sufficient to measure the lactate concentration. The amount of lactate was displayed in 60 seconds. All samples were measured using equipment made by the Japanese company Okra (Kyoto, Japan). The ROC was then used to determine the threshold concentration of lactate for measurement, and concentrations higher than 4.6 mM were considered to represent the rupture of membranes. The control group was selected from women who attended the clinic for a routine pregnancy check up without complaint of leakage. The women in the control group were matched for age with the women in case group. The evaluation of the amount of lactate in the vaginal fluid as well as the speculum testing, fern testing and nitrazine testing were performed in the control group using the same techniques used for the case group, and the results of both groups were compared.

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To analyze data, SPSS software version 17 was used. Frequency distribution tables, the mean and standard deviation, the Mann-Whitney test, the Chi-squared test, Fisher's exact test, and independent t-tests were used to describe and analyze the data. Statistical significance was defined as $p<0.05$.

Results

The study was conducted on 100 pregnant women, of whom 50 had PROM and 50 were healthy. The characteristics of the sample populations are shown in Table 1. The mean age of participants in the case group was 25.72±5.45 years, and the mean age in the control group was 25.74±6.21 years. Most of the participants in the case group (36%) had a high school diploma, and most of the control group (36%) had an elementary school education. Most of the participants in the case group (86%) and the control group (92%) were housewives. The husbands of most of the participants in the case group (52%) and the control group (75%) were self-employed (Table 1). Frequency distribution of women with PROM and women in the control group based on the demographic characteristics of pregnant women attending the Alavi Educational Therapeutic Center in Ardebil in 2010.

The mean gestational age was 37.51±2.51 weeks and 37.08±2.91 weeks in the PROM and control group, respectively. Because the distribution of the gestational age was not normal, according to Kolmogorov-Smirnov test, the Mann-Whitney test was used, and no significant difference was observed between the two groups with regard to gestational age.

The mean number of pregnancies was 1.24±1.22 and 1.68±0.86 in the case and the control groups, respectively. No significant difference

Table 1. Frequency distribution of women with PROM and women in the control group based on the demographic characteristics of pregnant women attending the Alavi Educational Therapeutic Center in Ardebil in 2010

Group	Group Prom	Group Control
Age	72±5/45/ 25 25	25/74±6/21
Education	(18) 36% High school school	(18) 36% Elementary School
Job **(housewife)**	(48)% 86	(46)% 92
Job **(husband) (Selfemploged)**	(26)% 52	(35)% 75

Table 2. Frequency distribution of women in the PROM and control groups based on the lactate concentration in the vaginal fluid of pregnant women attending the Alavi Educational Therapeutic Center in Ardebil in 2010

Group Lactat concentration	Group Prom		Group Control		Total	
	N	%	N	%	N	%
Positive	48	96	1	2	49	49
Negative	2	4	49	98	51	51
Total	50	100	50	100	100	100

was observed between the two groups. The mean number of miscarriages was 0.12± 0.24 and 0.26± 0.52 in the PROM and control groups, respectively. No significant difference was observed between the two groups. The mean number of deliveries was 1.01± 0.70 and 0.42± 0.86 in the PROM and control groups, respectively. No significant difference was observed between the two groups. No still births were reported in any of the groups.

The mean lactate concentration in the vaginal fluid was 8.39 mM and 1.99 mM in the PROM and control groups, respectively. After the threshold of 4.6 mM was determined using the ROC (Figure 1), 48 women (96%) in the PROM group were positive and 49 women (98%) in the control group were negative for the enzymatic staining of lactate in the vaginal fluids (Table 2).

Based on these data, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the lactate concentration in the vaginal fluid with a threshold of 4.6 mM for diagnosing PROM were 96%, 97.8%, 97.95%, 96.07%, and 97%, respectively.
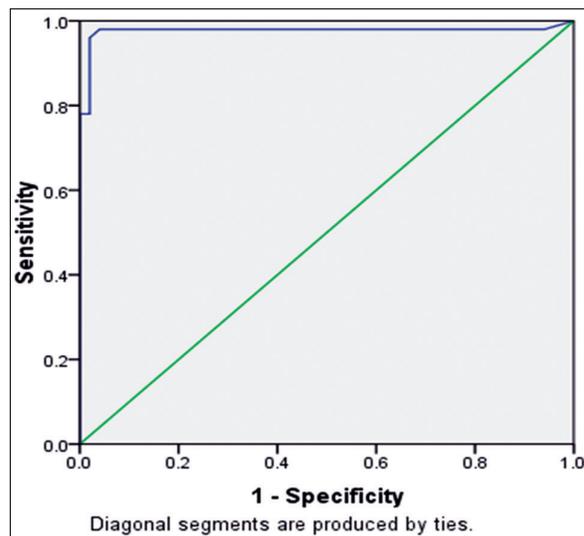

Figure 1. ROC of the lactate concentration in the vaginal fluid of pregnant women attending the Alavi Educational Therapeutic Center in Ardebil in 2010.

Discussion

In this study, the lactate concentration of the vaginal fluid was used to diagnose PROM. The present study showed that this technique has an acceptable diagnostic value. A method is diagnostically

acceptable only when its diagnostic indicators (sensitivity, specificity, positive predictive value, and negative predictive value) have values greater than 80% (10). Viberg-Itzel et al (2005) conducted a study to determine the optimal cut-off point for the lactate level to distinguish healthy membranes from ruptured membranes and to determine whether the lactate level in the vaginal fluid can be used as a diagnostic test when PROM is suspected. Viberg-Itzel et al. determined a cut-off point of 4.5 mM for the lactate concentration to achieve a sensitivity of 86%, specificity of 92%, positive predictive value of 92%, negative predictive value of 87%, kappa coefficient of 78%, and false negative of 15%. The results of the study of Viberg-Itzel et al. and the present study are similar, but the sensitivity, specificity, positive predictive value, and negative predictive value are higher in the present study. One of the reasons for this difference is the prospective nature of the study of Viberg-Itzel et al.; in contrast, our study was cross-sectional. Viberg-Itzel et al. excluded women who were suspected of PROM and enrolled only women who were positive for all 3 tests of speculum, fern, and nitrazine. Their control group consisted of women who were negative in all 3 tests. In the present study, 3 tests were used to confirm or exclude PROM. However, in the study of Viberg-Itzel et al., onlyspeculum test was used. The use of only one test to diagnose PROM increases the probability of error, which may result in reduced sensitivity and specificity.

The present study showed that the determination of the lactate concentration in the vaginal fluid is reliable, easy, consistent, and inexpensive in comparison with common diagnostic methods such as the nitrazine, fern, and speculum test. It is easier and less expensive than sonography, and it is easier, less expensive and less invasive than amniocentesis. Other methods that have been studied for the diagnosis of PROM include the measurement of vaginal D-amino oxydase, prolactin, alpha fetoprotein, insulin-like growth factor binding protein-1, human gonadotropin, fetal fibronectin, placental alpha macroglobulin 1, urea, creatinine, and thyroid hormones.

Park et al (2007) reported a sensitivity of 97–98%, specificity of 70–97%, and positive and negative predictive value of 98–100% for fetal fibronectin(19) .Lee et al (2007) reported a sensitivity of 98.7%, specificity of 87.5%, positive predictive value of 98.1%, and negative predictive value of 91.3% for placental alpha macroglobulin 1 (20).

Kariman et al (2006) reported a sensitivity of 95.3%, specificity of 97.7%, positive predictive value of 97.6%, negative predictive value of 95.5%, and accuracy of 96.5% for HCG using the ELISA method (21).

Kefali and Exalz (2007) and Kariman etal (2008) reported a sensitivity, specificity, positive predictive value, and negative predictive value of 100% for urea and creatinine (12, 22). It can therefore be inferred that the determination of the lactate concentration of the vaginal fluid is an easy, inexpensive, rapid, available, and reliable diagnostic test for PROM when compared with other tests.

Conclusion

The determination of the lactate level of the vaginal fluid is a reliable test with high sensitivity and specificity for PROM in pregnant women. Using a cut-off point of 4.6 mM and considering the PROM confirmation test as the gold standard for diagnosing PROM, the lactate concentration of the vaginal fluid has a favorable diagnostic value. Furthermore, compared with other diagnostic tests, it is reliable, easy, rapid, available and non-invasive.

References

1. *Cunningham F, Gant F, Leveno J. Williams Obstetrics. 23th Edition. New York. McGraw-Hill 2010.*

2. *Kaur A, Vats U, Nandanwar YS. Role of Serial Ultrasound Assessment in PROM Patientsand Its Outcome .Bombay Hospital Journal. 2009; 51 (2) :163-166.*

3. *Deering SH, Patel N, Spong CY, Pezzullo JC, Ghidini A .Fetal growth after preterm premature rupture of membranes: is it related to amniotic fluid Volume ? Matern, Fetal, Neonatal, Med. 2007; 20 (5): 397-400.*

4. Wiberg-Itzel E et al. Lactate Determination Inante- And Intrapartum Surveillance.Master Degree Department of Clinical Science and Education, Section of Obstetrics and Gynaecology (2007) Available at: http://diss.kib.ki.se

5. Esim E, Turan C, Unal O, Dansuk R,Cengizoglu B. Diagnosis of premature rupture of membranes by identification of beta-HCG in vaginal washing fluid. Eur J Obstet Gynecol Reprod Biol 2003; 107: 37-40.

6. Kayem G Maillard F. Rupture prematuree des membranes avant terme :attitude interventionniste ou xpectative ?Preterm premature rupture of membranes: Active orexpectant management?: Gynecologie Obstetrique & Fertilite. 2009; 37 (7) 334–341.

7. Torbe A, Czajka R. Are vaginal fluid procalcitonin levels useful for the prediction of subclinial infection in patients with preterm premature rupture of membranes. Obstet. Gynaecol. 2005; 31 (5) : 464–470.

8. Caughe B, Robinson N, Norwitz R. Contemporary Diagnosis and Management of Preterm Premature Rupture of Membranes. International Journal of Gynecology and Obstetrics.2008;1 (1) 11–22.

9. Healy AJ, Veille JC, Sciscione A, et al. The timing of elective delivery in preterm premature rupture of the membranes:a survey of members of the Society of Maternal-Fetal Medicine. Am J Obstet Gynecol. 2004;190:1479–1481.

10. Kim YH, Park YW, Kwon HS, Kwon JY, Him BJ.Vaginal fluid beta-Human chorionic gonadotropin level in the diagnosis of premature rupture of membranes. Acta Obstet Gynecol Scand. 2005;84(8):802-805.

11. Wiberg-Itzel E, Pettersson H, Cnattingius S, Nordstromd L. Prediction of time to spontaneous onset of labour with lactate concentration in vaginal fluid in women with suspected preterm prelabour rupture of the membranes. International Journal of Obstetrics and Gynaecology. 2009; 116 (1) :62-66.

12. Kafali H, Oksuzler C. Vaginal fluid urea and creatinine in diagnosis of premature rupture of membranes. Arch Gynecol Obstet 2007; 275(3): 157-160.

13. Cunningham F, Gant F, Leveno J. Williams Obstetrics. 22th Edition. New York. McGraw-Hill. 2005.

14. Gurbuz A, Karateke A, Kabaca C. Vaginal fluid creatinine in premature rupture of membranes. Int J ynaecol Obstet. 2004; 85(3): 270-271.

15. Akercan F, Cirpan T, Kazandi M. Terek MC, Mgoyi L, Ozkinay E. The value of the insulin-like growth factor binding protein-1 in the cervical-vaginal secretion detected by immunochromatographic dipstick test in the prediction of delivery in women with clinically unconfirmed preterm premature rupture of membranes. Eur J Obstet Gynecol Reprod Biol. 2005;121(2):159-163.

16. Wiberg-Itzel E, Pettersson H, Cnattingius S, Nordstromd L. Lactate determination in vaginal fluids: a new ethod in the diagnosis of prelabour rupture of membranes. BJ Obstet Gynaecol 2005; 112: 754-758.

17. Daneshmand SS, Cheung CY, Brace RA. Regulation of amniotic fluid volume by intramembranous absorption in sheep: role of passive permeability and vascular endothelial growth factor. Am J ObstetGynecol 2003;188:786 –793.

18. Nordstrom L, Achanna S, Naka K, Arulkumaran S. Fetal and maternal lactate increase during active second stage of labour. Br J Obstet Gynaecol 2001;108:263 –268

19. Park J, Lee Si, Norwitz ER. Non-invasive testing for rupture of the fetal membranes.Fetal &Maternal Healthcare US Obstetric and Gynecology 2007; (1): 13-16.

20. Lee SE, Park JS, Norwitz ER, et al. Measurement of placental alpha-microglobulin-1 in cervicovaginal discharge to diagnose rupture of membranes. Obstet Gynecol. 2007;109(3):634-640.

21. Kariman N, Jafari E, Amir MH, Alavi MH,The of cervicovaginal washing – fluid HCG for the diagnosis of premature rupture of membranes(PROM) in Iran in 2006.Research in Medicine 2007;31(1):43-47. [In Persian]

22. Kariman N,TolouiH, Azarhous R, Alavi MH, Diagnostic values of urea and creatinin values of cervicovaginal discharges in determining of premature rupture of membranes in Iran in2008..Research in Medicine 2010;33(4):222-228. [In Persian]

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