The Diagnostic Value of the Lactate Level in the Vaginal Fluid for Determining the Premature Rupture of Membranes

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Abstract

Objectives: To determine the diagnostic value of the lactate concentration in vaginal secretions in determining the premature rupture of membranes.

Subjects and Methods: One hundred 100 pregnant women with single pregnancy of gestational age 20–41 weeks were divided to two groups of 50 each. One group comprised of women with premature rupture of membranes, and the other (control group) of women with intact membranes. To verify the premature rupture of membranes in both groups, the speculum, fern, and nitrazine tests were done. Lactate Pro manual instrument was used to measure the lactate levels in vaginal fluid via enzymatic staining 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 lactate concentration of 4.6 mM in the vaginal fluid was diagnostic for premature rupture of membranes. It had a sensitivity of 96%, specificity of 98.8%, accuracy of 97%, positive predictive value of 97.9%, and negative predictive value of 96%.

Conclusions: Testing lactate levels in the vaginal fluid is an easy, rapid and reliable method for the diagnosis of premature rupture of membranes in pregnancy.

Key words: Premature rupture of membranes (PROM), vaginal fluids, lactate level.
Introduction

The premature rupture of membranes (PROM) is the spontaneous rupture of embryonic membranes before labor contractions at any gestational age that occurs in 2–25% of pregnancies. Its major complications are infections in mother and unborn child, umbilical cord prolapse, prenatal mortality, and premature labor. Premature rupture of membranes accounts for 30% premature labor and 18–20% prenatal mortality. Its accurate diagnosis is of importance because a false diagnosis can lead to unnecessary antibiotic therapy, corticosteroid therapy, or even labor induction. However, inability to diagnose PROM can lead to complications such as chorioamnionitis and pre-term labor. The diagnosis of PROM varies and is usually based on clinical evaluations such as observing fluid discharge during speculum testing, observing the fern model in microscopic tests, and biochemical tests. Among the biochemical tests, detection of nitrazine, vaginal di-amine oxidase, prolactin, alpha fetoprotein, insulin-like growth factor binding protein-1, human chorionic gonadotropin, fibronectin, and amniSure placental alpha macroglobulin-1 are used. Most of these tests have low sensitivity and specificity, or high false negative and false positive rates, or are invasive. Diagnosis on clinical findings fails in 10% cases. Speculum test has a false negative rate of 12%. 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. Its false negative rates range from 12.9 to 48.6%. False positive readings are caused by contamination with cervical mucus or semen as well as an incorrect sampling technique, and the false positive rates range from 5.8 to 30%. Recently, a new method has been devised for the diagnosis of PROM that relies on the measurement of lactate levels in the vaginal fluid. This method is easy to perform (can be performed at the 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 an indicator of tissue hypoxia. Lactate is mostly produced in the myometrium and/or chorio decidua and is transferred to the amniotic fluid through membranes. High concentrations of lactate (7–9 mM/l) can be detected in amniotic fluid, the amount of lactate in the amniotic fluid is 4–6 times higher than that in mother’s blood. Iberg-Iizel et al (2005) reported 86% sensitivity and 92% specificity of the lactate level in the vaginal fluids in determination PROM. The measurement of lactate concentration in the vaginal fluid is an easy, inexpensive, rapid, non-invasive, and available diagnostic method. Few studies have used this technique, therefore, the present study was conducted to determine the diagnostic value of lactate concentration in vaginal fluid for the determination of PROM in pregnant cases.

Subjects and Methods

One hundred pregnant women attending the pregnancy care clinic and midwifery emergency room of Alavi Educational Therapeutic Center in Ardebil, Iran were selected for the study. For selection convenient sampling method was used. 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 i.e those with ruptured membranes and those without rupture. Pregnant women with one fetus of gestational age of 20–41 weeks with main complaint of leakage who came to the emergency room of the Alavi Educational Therapeutic Center in Ardebil were included in the study. Exclusion criteria were known fetal abnormality, fetal asphyxia, fetal death, chronic diseases, 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 a sonogram before week 14, or have had two harmonious sonograms between 14 and 24 weeks. Written informed consent was obtained from all women. Questionnaire was filled and non-stress test (NST) test was taken for all women. To observe fluid leakage from the cervix, speculum testing was done in the lithotomy position. Based on the speculum test results, participants were divided to two groups of positive (PROM) and negative (intact membranes). Immediately after the speculum test, nitrazine test was performed using a swab 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. A swab was used in the same way to obtain 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. 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 paper strip that was already coated with the enzyme and attached to the Lactate Pro equipment. The amount of

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Lactate in the vaginal fluid was studied using enzymatic staining by Lactate Pro equipment. An aliquot of only 5 μl 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 receiver operating characteristic (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 any complaint of leakage. The women in the control group were matched for age with the cases and all tests were also done in them. The results of both groups were compared.

The data collected 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 expiry date of Japanese tapes (Okra, Japan) were 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 lactate concentration of the vaginal fluid, the inter observer method (kappa coefficient =0.86) was used.

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.

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Results

The study was conducted on 100 pregnant women, of them 50 had PROM and 50 were healthy. The mean age of participants in the case group was 25.74±5.21 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 cases (86%) and controls (92%) were housewives. The husbands of cases (52%) and controls (75%) were self-employed. The mean gestational age was 37.51±2.51 weeks in cases and 37.08±2.91 weeks in controls. 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.

The mean number of pregnancies (1.24±1.22 in the cases and 1.68±0.86 in controls), miscarriages (0.12±0.24 in cases and 0.26±0.52 in controls) and the mean number of deliveries (1.01±0.70 in cases and 0.42±0.86 in controls) were not significant. No still births was reported in any group.

The mean lactate concentration in the vaginal fluid was 8.39 mM in PROM cases and 1.99 mM in controls. A threshold of 4.6 mM was determined using the ROC (Figure), 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).

Table: 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.

<table>
<thead>
<tr>
<th>Lactate Concentration</th>
<th>PROM Group</th>
<th>Control Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Positive</td>
<td>48</td>
<td>96</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>4</td>
<td>49</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>

Figure: ROC of the lactate concentration in the vaginal fluid of pregnant women attending the Alavi Educational Therapeutic Center in Ardebil in 2010.
Based on the 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%, 98%, 96.1%, and 97%, respectively.

**Discussion**

In the present study, the lactate concentration of the vaginal fluid was used to diagnose PROM and a level of over 4.6 mM showed good correlation to PROM. A method is diagnostically acceptable only when its diagnostic indicators (sensitivity, specificity, positive predictive value, and negative predictive value) have values greater than 80%.

Viberg-Itzel et al. (2005) reported 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 present study are similar, but the sensitivity, specificity, positive predictive value, and negative predictive value are higher in the present study. One reason for this difference could be the prospective cross-sectional nature of our study. Viberg-Itzel et al. had excluded women who were suspected of PROM and enrolled only those 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, Viberg-Itzel et al. only used speculum test and this might have increased the probability of error, resulting 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 other common diagnostic methods such as the nitrazine, fern, and speculum test. Other methods that are used for the diagnosis of PROM include the measurement of vaginal D-amino oxidase, 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 97–98% sensitivity, 70-97% specificity and 98-100% positive and negative predictive value for fetal fibronectin. Lee et al. (2007) reported a 98.7% sensitivity, 87.5% specificity, 98.1% positive predictive value 91.3%, negative predictive value for placental alpha macroglobulin 1. 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. Kefali and Exalz (2007) and Kariman et al. (2008) reported a sensitivity, specificity, positive predictive value, and negative predictive value of 100% for urea and creatinine. Compared to all above tests, determination of lactate concentration in the vaginal fluid was found to be an easy, inexpensive, rapid, and reliable diagnostic test for the diagnosis of PROM.

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**References**

References