EVALUATION OF THE DIAGNOSTIC VALUE OF ASPARTATE AMINOTRANSFERASE AND ALANINE AMINOTRANSFERASE OF VAGINAL FLUID IN THE DIAGNOSIS OF PRETERM PREMATURE RUPTURE OF MEMBRANES

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ABSTRACT: Introduction: Premature rupture of membranes is one of the common problems in pregnant women and definitive diagnosis is of particular importance and this is important and necessary in suspected cases. The purpose of this study is to evaluate the diagnostic value of Aspartate aminotransferase and alanine aminotransferase of vaginal fluid in the diagnosis of preterm premature rupture of membranes. Methods: In this case-control study, 160 pregnant women with gestational age of 28 to 40 weeks (80 women with PROM as case group and 80 pregnant women without PROM as control group) were enrolled. Consecutive sampling was performed. The two groups were examined by speculum in terms of amniotic fluid and also were evaluated by Fern and Nitrazine test. Results: The mean age of the pregnant woman was 25.4 ± 5.6 years. Mean levels of aspartate aminotransferase and alanine aminotransferase of vaginal fluid was different in the two groups. This difference was statistically significant (P <0.001). Sensitivity and specificity of aspartate aminotransferase with a cut-off 7.5 IU / L were 79.7%, 56.2%, respectively and for the alanine aminotransferase with a cut-off 2.5 IU / L were 78.4% and 48.7%, respectively. Conclusion: the levels of aspartate aminotransferase and alanine aminotransferase in vaginal fluid were more in the cases of PROM than the cases of without PROM. The aspartate aminotransferase and alanine aminotransferase in vaginal fluid can be used as a predictive test for the diagnosis of PROM.

Keywords: Premature Rupture of Membrane, Aspartate Aminotransferase, Alanine Aminotransferase, Vaginal Fluid.

INTRODUCTION
Premature preterm rupture of membranes means the rupture of membranes before the onset of labor pain at gestational age less than 37 weeks (1). Premature preterm rupture of membranes is one of the most common problems which occurs in 30 to 40% of preterm births (2). Premature preterm rupture of membranes increases the maternal and fetal mortality rate that the associated complications such as umbilical cord prolapse, chorioamnionitis, Sepsis and preterm birth may occur (3,4).

Since many fetal complications are caused by prolonged rupture of membranes, rapid diagnosis of PROM is very important (5). Diagnosis of PROM is currently carried out by the following methods; 1-the mother's history of the sudden departure of fluid from vagina and direct observation of clear fluid in the posterior fornix of the
vagina or leakage of fluid from the mouth cervix through examination by sterile speculum (6), 2-Nitrazine test which examines the alkaline PH of cervicovaginal secretions. The test has high rate of false positive in the cases of cervicitis, vaginitis, alkaline urine, semen or blood contamination and the use of antiseptics (7, 8). 3- Fern test in which microscopic crystallization examination of amniotic fluid is done. The false positive is performed for contamination with cervical mucus and semen and false negative in test wrong technique or contamination with blood (9-11). 4- Amnio-dye test, the test includes Amniocentesis and injecting dye into the amniotic fluid. The definitive diagnosis for PROM is leakage of colored fluid in the vagina after 20 to 30 minutes and observing the tampon infected with this color. This is an invasive procedure and associated with the risk of bleeding, placental infection, iatrogenic rupture of amniotic sac and abortion. 5- Ultrasound alone cannot be a diagnostic tool, but it may help to diagnose. 6- Amni sure is a simple and rapid and less invasive method which evaluates placental alpha-I microglobulin in the vaginal fluid, but its cost is high (12).

Diagnosis of PROM is very difficult after 48 hours or in long and preterm rupture of membranes and in most cases, the negative results are obtained from Nitrazine or Fern test. Since the lack of diagnosis can lead to serious pregnancy complications, proper diagnosis is required (13). For these reasons, a non-invasive, simple, inexpensive and available method for is required the diagnosis of rupture of membranes. Several markers including Alpha-fetoprotein, Fetal Fibronectin, creatinine, urea, prolactin and placental beta subunit have been studied for this purpose (14-17).

The studied evidence suggests that liver enzymes including aspartate aminotransferase and alanine aminotransferase are produced in the amniotic fluid by the fetus from the second half of pregnancy. These levels in amniotic fluid are not related to these levels in serum of mother. (5). Therefore, in this study, the rate of aspartate aminotransferase and alanine aminotransferase obtained from washing the vaginal fluid were evaluated in the diagnosis of PROM and determining the cut-off point.

MATERIALS AND METHODS
This case-control study was performed at Ali ibnAbiTaleb hospital in April 2013 to August 2014 in the city of Zahedan, Sistan and Baluchestan province, Iran, Zahedan University of Medical Sciences, after approval by the Ethics Committee of Zahedan University of Medical Sciences. 160 pregnant women with gestational age 28 to 40 weeks in two groups were enrolled. Group 1: 80 pregnant women who had diagnosis of premature rupture of membranes at gestational age of 28 to 40 weeks. Group 2: 80 pregnant women referred to Obstetrics clinic for periodic check-up and had a gestational age of 28 to 40 weeks. Gestational age was determined based on the first day of the last menstrual period and first trimester ultrasound. Rupture of membranes was confirmed with examination by sterile speculum and observation of cervical fluid leakage or accumulation of fluid in the posterior fornix of the vagina and positive Fern and Nitrazine test. The patients with congenital malformations, fetal growth restriction, fetal distress, placenta previa, vaginal bleeding, vaginal infection, maternal disease, pregnancy hypertension, preeclampsia, and complications of pregnancy were excluded from the study.

In all patients, 5 cc sterile normal saline was poured into the posterior fornix of the vagina by syringe 5cc, and after a few minutes, the liquid was aspirated by the same syringe and was sent the laboratory of Zahedan Ali ibnAbiTaleb hospital and was centrifuged for 10 minutes. Alcyon automatic set and commercial kits were used to measure the concentrations of aspartate aminotransferase and alanine aminotransferase.

Mann Whitney U Test was used to compare the levels of aspartate aminotransferase and alanine aminotransferase in two groups, and ROC curve was drawn for the levels of aspartate aminotransferase and alanine aminotransferase, and the sensitivity and specificity were calculated and the cut-off point was determined at the highest desirable sensitivity and specificity. Also,SPSS software(version 16) was used to analyze the results.

RESULTS
There was no significant difference between two groups of with PROM and without PROM in terms of demographic characteristics. The results are shown in Table 1.
Table 1: Comparison of demographic characteristics between two groups of with PROM and without PROM

<table>
<thead>
<tr>
<th>Variables</th>
<th>(Mean±SD) Case (n=80)</th>
<th>Mean±SD Control (n=80)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>25.0±5.6</td>
<td>25.8±5.5</td>
<td>0.368</td>
</tr>
<tr>
<td>Gestational age by sonography</td>
<td>36.4±2.5</td>
<td>35.7±2.4</td>
<td>0.071</td>
</tr>
<tr>
<td>Parity (N)</td>
<td>2.4±1.7</td>
<td>2.7±1.6</td>
<td>0.245</td>
</tr>
<tr>
<td>Gravid (N)</td>
<td>1.2±1.5</td>
<td>1.4±1.4</td>
<td>0.460</td>
</tr>
<tr>
<td>Abortion (N)</td>
<td>0.21±0.54</td>
<td>0.32±0.63</td>
<td>0.229</td>
</tr>
</tbody>
</table>

Analysis was performed by Independent T-test.
There was a significant difference between two groups in terms of aspartate aminotransferase and alanine aminotransferase levels which is shown in Table 2.

Table 2: Comparison of the levels of aspartate aminotransferase and alanine aminotransferase in the samples obtained from vaginal washing between two groups of with PROM and without PROM

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>ALT</th>
<th>AST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Group with PROM</td>
<td>5.0±4.8</td>
<td>16.5±12.3</td>
</tr>
<tr>
<td>Group without PROM</td>
<td>3.3±2.6</td>
<td>9.1±7.9</td>
</tr>
<tr>
<td>P Value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Analysis was performed by Mann Whitney U Test.
Based on the results of the study, the sensitivity and specificity and positive and negative predictive values for aspartate aminotransferase were obtained 79.7%, 56.2%, 64.2%, 73.7%, and for alanine aminotransferase were obtained 78.4%, 48.7%, 60.1%, 69.6%. In this study, the best cut-off point for aspartate aminotransferase was 7.5 IU / L and for alanine aminotransferase 2.5 IU / L. (ROC Curves 1,2)

ROC curve No. 1: aspartate aminotransferase
DISCUSSION

In this study, there was a significant difference between two groups of with PROM and without PROM in terms of the levels of aspartate aminotransferase and alanine aminotransferase. The study of Kale and colleagues in 2008 was performed on 84 women (36 pregnant women with a diagnosis of preterm premature rupture of membrane (PPROM) and 48 pregnant women without PPROM). Levels of alanine aminotransferase and aspartate aminotransferase were compared in the two groups and the levels of alanine aminotransferase was higher than in the group with PROM than the group without PROM (13.35 ± 5.27 vs. 1.30 ± 0.93 and P=0.064).

Aspartate aminotransferase concentration in vaginal fluid was significantly higher in the group with PROM than the group without PROM (17.46 ± 14.4 vs. 7.8 ± 3.08 and P=0.001), and desirable cut-off point 3, sensitivity 91%, specificity 83%, positive predictive value 80% and negative predictive value 93% were achieved (5).

In a study by Ali farid et al, about the levels of aspartate aminotransferase and alanine aminotransferase of the vaginal fluid in 2011, 45 pregnant women with PROM and 45 pregnant women without PROM were compared. Gestational age was 26 to 36 weeks. Aspartate aminotransferase and alanine aminotransferase levels were significantly higher in the group with PROM than the group without PROM (P<0.001). Cut-off point for aspartate aminotransferase was 1.25 IU / L and sensitivity 97.8% and specificity 62.2% and negative predictive value of 96.55% were obtained, and the cut-off point for alanine aminotransferase was 0.5 IU / L and sensitivity 87.7% and specificity of 62.2%, negative predictive value 85% and positive predictive value 78% were achieved (18).

In our study, the sensitivity and specificity of aspartate aminotransferase and alanine aminotransferase were lower than other studies, but the studied population in our study was larger than the other studies.

Kale Ebro and colleagues reported that alanine aminotransferase levels in vaginal fluid was not significantly different between two groups (with PROM and without PROM), but aspartate aminotransferase levels in vaginal fluid was significantly different, and its sensitivity and specificity, and positive and negative predictive values were 91%, 83%, 80%, and 93%, respectively (5).

In the study of Asgharnia and colleagues in 2014 that aimed to identify the predictive value of aspartate aminotransferase and alanine aminotransferase of vaginal fluid for diagnosis of PROM, 148 pregnant women were studied and the results showed that the levels of alanine aminotransferase was not significantly different between two groups, but the levels of aspartateaminotransferase was significantly different between two groups (19). In our study, the difference was observed in the two enzymes. Due to the small number of studies, it seems that further studies with a larger population should be performed on the aspartate aminotransferase and alanine aminotransferase.
aminotransferase levels of vaginal fluid and it is needed to determine cut-off point for PROM at different gestational ages. The test is simple, fast, inexpensive, and commercial kits are available and can be done in most hospitals at all hours of the day and is economically cheaper than other tests. Thus, the levels of aspartate aminotransferase and alanine aminotransferase of vaginal fluid can be used as convenient and fast reliable test for the diagnosis and prediction of PROM.

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