Aspartate Aminotransferase and Alanine Aminotransferase in Vaginal Fluid for Detecting Preterm Premature Rupture of Membranes

Ali Farid, A. ; Noha Rabei,A. ; Mohamed Taha and Ahmed Abdel Shafy

Department of Obstetrics and Gynecology, School of Medicine, Ain Shams University, Cairo, Egypt
Tatto_cairo_egypt@yahoo.com

Abstract: Objectives: To determine if the level of vaginal fluid aspartate aminotransferase (AST) and alanine aminotransferase (ALT) is of value in the diagnosis of preterm premature rupture of membrane (PPROM). **Patients and methods**: Ninety patients were included in this study: 45 patients with PPROM and 45 patients as controls. The gestational age ranged from 26 to 36 weeks. AST and ALT in vaginal fluid were measured in both groups. **Results**: The vaginal AST and ALT were highly significant($p < 0.001$) in cases when compared to the controls. At AST cutoff value of 1.25 IU/L the sensitivity was 97.8% and specificity was 62.2%, and negative predictive value was 96.55% so it can be used as a good predictive test for detection of PPROM. At ALT cutoff value of 0.5 iu/L the sensitivity was 86.7%, specificity 75.6%, positive predictive value 78% and negative predictive value 85%. **Conclusion**: Vaginal AST and ALT could be used as an excellent predictive test for detection of PPROM.
[Ali Farid, A. ; Noha Rabei, A.; Mohamed Taha and Ahmed Abdel Shafy **AspartateAminotransferase and Alanine Aminotransferase in Vaginal Fluid for Detecting Preterm Premature Rupture of Membranes**] Journal of American Science 2011; 7(11):243 -247].(ISSN: 1545-1003). http://www.americanscience.org

Keyword: Aspartate, Aminotransferase, Premature Rupture, Membranes.

1. Introduction

Preterm premature rupture of membranes (PPROM) refers to rupture of the fetal membranes before the onset of labor at less than 37 weeks gestation (Song and Han, 2005). It complicates only 2-3% of pregnancies but is associated with 40% of preterm deliveries and can result in significant neonatal morbidity and mortality (Douvas et al., 1984; Maxwell, 1993; Merenstein et al., 1996; Helmer, 2006).

The diagnosis is made by a history suggestive of spontaneous rupture of membranes followed by a sterile speculum examination demonstrating pooling of fluid in the posterior vaginal fornix. Ultra-sound examination demonstrating oligohydramnios is also used to help confirm the diagnosis of spontaneous rupture of the membranes (Carlan et al., 1993; Carroll et al., 1995; Combs et al., 2004).

A series of tests have been used to confirm membranes rupture; the most widely used has been the nitrazine test, which detects pH change. Unfortunately, nitrazine paper testing of vaginal pH has an appreciable false-positive rate associated with blood contamination, semen, or bacterial vaginosis. Diagnosis of PPROM is difficult when maternal history of PPROM is not supported by vaginal pooling of amniotic fluid or membrane rupture is slight (Cunnigham and Gant, 2001).

These potential limitations have led to the search for biochemical markers for the detection of PPROM. Among the markers evaluated were HCG in vaginal washing fluid, prolactin and calcitropic hormones, and insulin-like growth factor binding protein-1 in the cervical-vaginal secretion, but these biochemical markers have limited success rate for the detection of PPROM (Esim et al., 2003; Shaarawy and El-Minawi, 2004; Akercan et al., 2005; Kale et al., 2008).

Amniotic fluid in the second half of human gestation is largely a product of fetal urine and an additional source of amniotic fluid are respiratory and gastrointestinal tract excretions (Muller et al, 1994; Fauza, 2004). Evidence suggests that liver enzymes of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are produced by the fetus. These levels do not correlate with maternal levels (Kale et al., 2008) and their concentrations in the amniotic fluid have been shown by different studies (Kuczynska-Sicinska et al., 1989; Smolarczyk et al., 1996; Kale et al., 2008).

Objective:

To determine whether measurement of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in vaginal fluid is useful for the diagnosis of preterm premature rupture of membranes (PPROM).

2. Patients and Methods

Type of the study: Case Control study

This study was carried out in Ain Shams University Maternity Hospital in the time between January to December 2009.

Ninety pregnant women were included in the study between 26 and 36 weeks of gestation divided into two groups:

Group I: 45 pregnant women admitted to the hospital clinically diagnosed as PPROM (n= 45, study group) at gestational age between 26-36wk.

Group II: 45 normal pregnant women (n= 45, control group) attending the outpatient clinic for antenatal care at the same gestational age.

The gestational age was estimated according to the last menstrual period and confirmed by a first trimesteric ultrasound. If menstrual history was unreliable, the ultra-sonographic date would be used. Rupture of membranes was diagnosed by sterile speculum examination confirming fluid leakage from the cervical canal or pooling of fluid in the posterior vaginal fornix.

Patients with congenital fetal malformations, fetal growth restriction, fetal distress, placenta previa, vaginal bleeding, pregnancy induced hypertension and pre-eclampsia were excluded from the study.

Methodology:

In PPROM patients, vaginal fornix was irrigated with 3ml of sterile saline using a 5-ml syringe, the fluid was aspirated from the posterior vaginal fornix using the same syringe The fluid specimens were collected to polypropylene tubes then immediately centrifuged for 10min. For measurement of AST and ALT concentrations a photometric method was applied by automated machine HITACHI 912 in Ain Shams University lab, using commercial kits (BM Egypt).

After confirming the absence of amniotic fluid pooling and blood in the posterior fornix for the control group, the sampling method and AST and ALT assays were performed as previously described for PPROM pregnant women.

3. Results

There was no statistically significant difference between cases and controls as regards demographic data as shown in table (1).

AFI was lower in PPROM group than in control. On the other hand TLC was higher in cases than in controls, the differences were statistically highly significant. There was no statistically significant difference between cases and controls as regard fetal weight. This is shown in table (2).

Vaginal AST and ALT were lower in controls than cases and the differences were highly statistically significant (p <0.001) as shown in table (3).

Mann-Whitney's U-test was used as a descriptive parameter for vaginal AST and ALT due to non parametric distribution owing to presence of extreme values.

There was a significant positive correlation between vaginal ALT and AST especially at lower values as shown in table (4) and figure (1).

There was statistically significant negative correlation between vaginal ALT and AFI (r=-0.528, p <0.001) and a statistically significant good positive correlation between vaginal ALT and TLC (r= 0.456, p <0.001).

There was statistically significant positive correlation between vaginal AST and both TLC (r= 0.527) and gestational age (r= 0.264, p<0.001). There was statistically significant good negative correlation between vaginal AST and AFI (r= -0.593, p<0.001).

On correlating AFI and TLC (10^3/ML), there was a statistically highly significant negative correlation (p=0.001) and the negative r-value indicates that the less AFI would be the higher TLC (10^3/ML) was as shown in table (5).

Table (1): Comparison between cases and controls as regard demographic data*

	Cases (n=45)	Controls (n=45)	t	p
Age (yrs)	27.42±5.64	25.81±2.65	1.73	0.087 NS
Gestational age by U/S	31.91±2.87	30.89±2.13	1.9	0.06 NS
Deliveries (n)	1.28±1.45	1.68±0.84	-1.59	0.116 NS
Abortions (n)	0.44±0.81	0.51±0.58	-0.44	0.657 NS
BMI (kg/m2)	26±2.37	25.26±2.11	1.63	0.106 NS

*Independent t-test. Data are presented as mean ± standard deviation.
NS=non significant, S=significant and HS=highly significant

Table (2):Comparison between cases and controls as regard investigations results*

	Cases(n=45)	Controls(n=45)	t	p
AFI (cm)	6±2.54	15.22±2.49	-17.4	0.0001 HS
TLC (10^3/ML)	11.74±3.46	6.4±1	9.886	0.0001 HS
Fetal weight (kg) by U/S	2.21±0.64	2.23±0.56	-.113	0.910 NS

*Independent t-test. Data are presented as mean ±standard deviation.
NS=non significant, S=significant and HS=highly significant.

Table (3): Comparison between cases and controls as regard vaginal AST (IU/l) and Vaginal ALT (IU/l) levels*

	Cases (n=45)	Controls (n=45)	p
Vaginal AST (IU/L)	18(11-34)	0 (0-3)	0.001 HS
Vaginal ALT (IU/L)	4(1-10)	0(0-1)	0.001 HS

* Mann-Whitney's U test
Data were expressed as median (interquartile range)

Table (4):Correlation between vaginal ALT (IU/L) and vaginal AST (IU/L) in the population of the study.

		Vaginal AST (IU/L)
Vaginal ALT (IU/l)	*r-value*	0.888
	p-*value*	<0.001 HS*

* Highly significant test P <0.01.

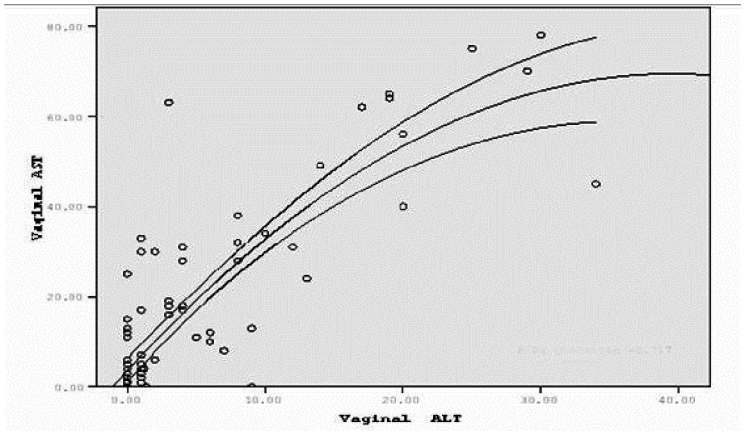

Fig. (1): Scatter plot showing the significant positive correlation between vaginal ALT and AST specially at lower values

At AST cutoff value of 1.25 IU/l the sensitivity was 97.8% and specificity was 62.2%. The positive predictive value was 72.3% and negative predictive value was 96.55%. The likelihood ratio (LR) for a positive test was 2.59 and negative likelihood ratio (LR) was 0.04, we see that AST can be used as a good predictive test for detection of PPROM.

At ALT cutoff value of 0.5 IU/l the sensitivity was 86.7% and specificity was 75.6%. The positive predictive value was 78% and negative predictive value was 85%. The likelihood ratio (LR+) for a positive test was 3.5 and negative likelihood ratio (LR-) was 0.18.

4. Discussion

PROM occurs in 10% of all pregnancies. Preterm prelabor rupture of the membranes (PPROM) occurs in approximately 2% of all pregnancies ***(Cox et al., 1988)***.

The most frequent consequence of PPROM is preterm delivery, accounting for up to one third of preterm deliveries. In addition early PPROM may cause neonatal pulmonary hypoplasia leading to an increased risk of neonatal death, even if delivery occurs at gestational age at which the outcome would usually be good ***(Bennet, 2007)***.

In addition, PPROM poses several maternal risks including the risk of chorioamnionitis, placental abruption, risks of drugs taken (steroids, antibiotics and tocolytics) as well as puerperal endometritis ***(Svigos et al., 2006)***.

The most reliable method for diagnosing PPROM is visualizing amniotic fluid draining through the cervix. However, certain diagnosis of PPROM is sometimes difficult. False diagnosis of PPROM may lead to limit unnecessary obstetric interventions, including hospitalization, administration of antibiotics and corticosteroids, and even induction of labor, while delayed diagnosis of PPROM may worsen the adverse consequences that may complicate PPROM ***(Hannah et al ., 2000, Healy et al., 2004)***.

Several suggestions have been made for methods to confirm the diagnosis of PPROM when in doubt. These included ultrasound assessment of amniotic fluid volume, intraamnionitic injection of dye, pH testing of the vaginal fluid, and physical characteristics of the vaginal fluid itself (e.g. ferning). This forced the research to look for a biochemical

marker solely secreted in the amniotic fluid that may be detected in vaginal fluid in cases of PPROM. Studies on several markers have been conducted including fibronectin, prolactin, HCG, AFP and other markers. Nevertheless, none of these methods reached a satisfactory diagnostic accuracy making one of them a reliable method for confirming the diagnosis of PPROM ***(Mercer, 2004)***.

Evidence suggests that AST and ALT are produced by the fetus and measured in detectable levels in amniotic fluid. These levels were not shown to correlate with maternal serum AST and ALT ***(Kale et al., 2008)***.

The aim of the current study was to determine whether measurement of AST and ALT levels in vaginal fluid is useful for the diagnosis of preterm premature rupture of membranes (PPROM).

In this study the vaginal fluid AST concentration was significantly higher in women with PPROM compared to women of the control group 18(11-34) IU/L vs. 0 (0-3) IU/L, respectively, $p<0.001$). Vaginal fluid ALT concentration was significantly higher in women with PPROM compared to women of the control group 4(1-10) IU/L vs. 0 (0-1) IU/L, $p<0.001$, respectively). There was statistically significant good negative correlation between vaginal ALT and AFI ($r=-0.528$, $p<0.001$) and a statistically significant good positive correlation between vaginal ALT and TLC. ($r= 0.456$, $p<0.001$).

There was statistically significant good positive correlation between vaginal AST and both TLC (r-value= 0.527) and gestational age ($r= 0.264$, $p<0.001$). There was statistically significant good negative correlation between vaginal AST and AFI ($r= -0.593$, $p<0.001$).

The best cutoff value for vaginal AST concentration for detection of PPROM was 1.25 IU/l (sensitivity 97.8%, specificity 62.2%, PPV 72.3%, NPV 96.55%, the positive likelihood ratio (LR+) was 2.59 and the negative likelihood ratio (LR-) was 0.04) while the best cutoff value for vaginal ALT concentration for detection of PPROM, was 0.5 IU/l (sensitivity 86.7%, specificity 75.6%, the positive predictive value 78% and negative predictive value 85%, the positive likelihood ratio (LR+) was 3.5 and negative likelihood ratio (LR-) was 0.18.

Kale et al. (2008) conducted a study that included 84 women. 36 women with a diagnosis of PPROM (at 26-36 weeks' gestation) were compared to 48 women as control group concerning vaginal AST and ALT concentrations. Vaginal fluid ALT concentration was slightly higher in women with PPROM compared to women of the control group (5.27±13.35 U/l vs. 0.93±1.30 U/l, respectively, $p=0.064$). Yet, this difference did not reach statistical significance. However, vaginal fluid AST was significantly higher in women with PPROM (14.4±17.46 U/l vs. 3.08±7.8 U/l, respectively, $p=0.001$). The optimal AST cutoff value found by ***Kale et al. (2008)*** was 3 IU/l (sensitivity level of 91% at a specificity of 83%, with positive and negative predictive values of 80 % and 93%, respectively). They concluded that AST levels >3 IU/l, when present between 26 and 36 weeks' gestation, can identify PPROM in approximately 91% of women. They speculated that this test had a high negative predictive value for PPROM; its use could at least limit unnecessary patient hospitalization.

We concluded that vaginal AST and vaginal ALT could be used as an excellent predictive test for detection of PPROM. So, this could limit unnecessary obstetric interventions, including hospitalization, administration of antibiotics and corticosteroids, and even induction of labor and iatrogenic prematurity. It is rapid, easy performed test as the commercial kits are available in most of hospital labs with reasonable cost.

References

1. Akercan F, Cripan T and Tarek MC(2005): The value of insulin like growth factor binding protein 1 in the vaginal and cervical secretions in predict-tion of delivery in patients with PROM. Eur J Obstet Gynecol Reprod Biol.; 1:121.
2. Bennett Phillip(2007): Preterm labour. Dewhurst's text-book of Obstetrics and Gynecology.; 21: 177.
3. Carlan SJ, O'Brien WF, Parsons MT, Lense JJ(1993): Preterm premature rupture of membranes. A randomised study of home versus hospital management. Obstet Gynecol.; 81: 61-4.
4. Carroll SG, Papiaoannou S, Nicolaides KH(1995): Assessment of fetal activity and amniotic fluid volume in the prediction of intrauterine infection in preterm prelabour amniorrhexis. Am J Obstet Gynecol.; 172:1427-35.
5. Combs CA, McCune M, Clark R, Fishman A.(2004): Aggressive tocolysis does not prolong pregnancy or reduce neonatal morbidity after-preterm premature rupture of the membranes. Am J Obstet Gynecol.; 190: 1728-31.
6. Cox SM, Williams ML, Leveno KJ(1988): The natural history of preterm ruptured membranes: What to expect of expectant management. Obstet Gynecol.; 71: 558-562.
7. Cunningham FG and Gant NF(2001): Williams Obstetrics. New York, McGraw-Hill, 2001, p 707-708.
8. Douvas SG, Brewer JMJ, McKay ML, Rhodes PGJ, Kahlstorf JH, Morrison JC(1984): Treatment

of preterm rupture of the membranes. J Reprod Med.; 29: 741-4.
9. 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.
10. Fauza D(2004): Amniotic fluid and placental stem cells. Best Pract. Res Clin Obstet. Gynaecol.; 18:877-91.
11. Hannah ME, Hodnett ED, Willan A, *et al.* (2000):The Term PROM Study Group, authors. Prelabor rupture of the membranes at term: expectant management at home or in hospital? Obstet Gynecol.; 96: 533-538.
12. Healy AJ, Veille JC, Sciscione A, *et al.* (2004):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.; 190: 1479-1481.
13. Helmer H(2006): Continuing challenges in treating preterm labour: rupture of the membranes. BJOG; 113: 111-12.
14. Kafali H and Oksuzler C(2007): Vaginal fluid urea and creatinine in diagnosis of premature rupture of membranes. Arch. Gynecol. Obstet.; 275: 157-60.
15. Kale A, Kale E, Akdeniz N, Kuyumcuoğlu U, Canoruc N(2007): Proposal of a diagnostic test for the detection of preterm premature rupture of membranes based on the determination of thyroid hormones in vaginal washing fluid. Fetal Diagn Ther.; 22 (5): 330-4.
16. Kale Ebru, Umur Kuyumcuoglu, Ahmet Kale, Ali Erfan, Naime Canoruc (2008): A New and practical Aspartate Aminotransferase Test in vaginal washing fluid. Fetal Diag Therapy; 24:425-28.
17. Kuczynska-Sicinska J, Wojcicka-Jagodzinska J, Romejko E, Smolarczyk R, Siekierski BP, Chodzinska B(1989): Biochemical studies of the amniotic fluid in arterial hypertension in relation to intrauterine growth retardation. I. Parameters of the proteins, lipids, enzymes and renal maturity. Ginekol Pol.; 60: 266-70.
18. Lockwood, CJ, Senyei, AE, Dische, MR, *et al.(*1991):. Fetal fibronectin in cervical and vaginal secretions as a predictor of preterm delivery. N Engl J Med.; 325: 669.
19. Maxwell GL(1993): Preterm premature rupture of membranes. Obstet Gynecol Surv.; 48: 576-83.
20. Mercer BM(2004): Preterm premature rupture of the membranes diagnosis and manage. Obstet Gynecol Clin Perinatol.; 31:765-82.
21. Merenstein GB and Weisman LE(1996): Premature rupture of the membranes: neonatal consequences. Semin Perinatol.; 20: 375-80.
22. Muller F, Dommergues M, Ville Y, Lewin F, Delvalez- Morichon N, Nihoul-Fekete C, Bargy F, Dumez Y, Boue A(1994): Amniotic fluid digestive enzymes: diagnostic value in fetal gastro-intestinal obstructions. Prenat Diagn.; 14: 973-79.
23. Shaarawy M and El-Minawi AM(2004): Prolactin and calcitropic hormones in preterm premature rupture of mem-branes. Int J Gynaecol Obstet.; 84: 200-207.
24. Smolarczyk R, Wojcicka-Jagodzinska J, Romejko E, Czajkowski K, Piekarski P, Teliga-Czajkowska J, Kuczynska-Sicinska J (1996): Evaluation of fetal condition in pregnancy complicated by hypertension, biochemical assessment of amniotic fluid. Enzymes. Ginekol Pol.; 67: 598-602.
25. Song AG and Han SM(2005): Effect of antenatal corticosteroids and antibiotics in pregnancies complicated by premature rupture of membranes. J Korean Med Sci.; 20: 88-92.
26. Svigos John Michael, Jeffrey S. Robinson, Rasniah Vigneswaran(2006): High Risk Pregnancy: Manage-ment Options; 3rd ed., Chapter 63-Prelabor Rupture of the Membranes,.

11/25/2011