

ASPARTATE AMINOTRANSFERASE AND ALANINE
AMINOTRANSFERASE IN VAGINAL FLUID FOR DETECTING
PRETERM PREMATURE RUPTURE OF MEMBRANE

دور مستويات أنزيم ناقل أمين الأسبارتيك AST وأنزيم ناقل أمين الألانين ALT
في السائل المهبل في تشخيص حالة تمزق الأغشية المبكر

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ملخص البحث

هدف البحث: يحدث تمزق الأغشية المبكر في 2% من حالات الحمل، كما أنه السبب في 30-40% من حالات الولادة المبكرة، لذا فإن التشخيص المبكر لحالات تمزق الغشاء المبكر يحتل أهمية خاصة لما له من انعكاسات كبيرة على نتائج الحمل، كما أن التشخيص الخاطئ لهذه الحالة قد يقود لإجراء تدخلات توليدية لا لزوم لها. تهدف هذه الدراسة إلى تحديد القيمة التشخيصية لمستوى أنزيم ناقل أمين الأسبارتيك AST وأنزيم ناقل أمين الألانين ALT في السائل المهبل في تشخيص تمزق الأغشية المبكر PROM، كاختبارات غير راضية ومتاحة.

طرق البحث: تم إجراء دراسة مقطعية وصفية مستقبلية في قسم الأمراض النسائية والتوليد في مدينة الإمامين الكاظميين عليهما السلام في بغداد في الفترة ما بين شهر آب 2015 وحتى شهر أيار 2016. تم تضمين 100 امرأة حامل بين الأسبوعين 28 و36 من الحمل في هذه الدراسة. عانت 50 مريضة من حالة تمزق أغشية مبكر PROM اعتبرن مجموعة الدراسة، بينما لم يلاحظ تمزق باكر عند 50 امرأة أخرى مثلن مجموعة المقارنة. تم قياس مستويات AST وALT في السائل المهبل في كلتا المجموعتين، ثم استخدام اختبار Mann Whitney U لمقارنة مستويات AST وALT بين المجموعتين.

النتائج: بلغ متوسط مستوى AST في السائل المهبل في مجموعة الدراسة 8.8 (5.5-17.2 وحدة دولية/ل)، مقابل 5.1 (2.9-8.2 وحدة دولية/ل) في مجموعة المقارنة ($p < 0.001$). بلغت حساسية ونوعية أنزيم AST بالنسبة للتشخيص 80% و58% على الترتيب. من جهة أخرى فقد بلغ متوسط مستوى أنزيم ALT في مجموعة الدراسة 6.9 (4.5-15.2 وحدة دولية/ل) و4.6 (3.1-7.7 وحدة دولية/ل) في مجموعة المقارنة ($p < 0.004$)، بلغت حساسية ونوعية أنزيم ALT بالنسبة للتشخيص 78% و48% على الترتيب.

الاستنتاجات: وفقاً لنتائج هذه الدراسة، فقد كانت مستويات AST وALT في السائل المهبل أعلى في حالات وجود تمزق أغشية باكر PROM مقارنة بحالات عدم وجوده، وبذلك فإن مستويات أنزيمات AST وALT في السائل المهبل يمكن أن تستخدم بمثابة اختبار لتشخيص حالة تمزق الأغشية المبكر.

ABSTRACT

Objective: Premature rupture of membranes (PROM) occurs in 2% of pregnancies, and 30-40% of preterm

labors are related to this problem. Early diagnosis of PPRM is very important due to its impact on pregnancy outcomes, false diagnosis of PPRM may lead to unnecessary obstetric interventions. The aim of this

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study was to determine the diagnostic value of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in vaginal fluid for the diagnosis of preterm premature rupture of membranes as a non-invasive and available test.

Methods: A total of 100 pregnant women between the 28th-36th gestational weeks were enrolled in the study. Fifty patients were in PPRM group and 50 in control group. AST and ALT levels in vaginal fluid were measured in each group. Mann Whitney U-test was used to compare AST and ALT levels in each group.

Results: The mean of AST level in vaginal fluid was 8.8 (5.5-17.2 IU/L) in PROM group versus 5.1 (2.98.2- IU/L) in control group ($p < 0.001$), sensitivity and specificity for AST was 80%, 58% respectively, while the ALT levels in PPRM group was 6.9 (4.5-15.2 IU/L) and in control group 4.6 (3.1-7.6 IU/L) ($p < 0.004$), the sensitivity and specificity was 78%, 48% respectively.

Conclusions: According to the findings of this study, the levels of AST and ALT in vaginal fluid were higher in the cases of PPRM than in the cases without PPRM. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in vaginal fluid could be used as a predictive test for the diagnosis of PPRM.

INTRODUCTION

Premature rupture of membrane (PROM) is common clinical problem, occurrence with an incidence of 2% of all pregnancies.¹ It is a significant event as it cause maternal complications, increased operative procedures, neonatal morbidity and mortality.²

The management of a case of PROM has remained as one of the most difficult and controversial problems in obstetrics over the past several decades.² The management of PROM has gone through various cycles of obstetric activity from benign neglect to immediate intervention. Paralleling these cycles of activity there have been varying degrees of concern about infection.² Mean while incidence has remained unabated and is still responsible for large number of neonatal mortality.²

Premature rupture of membrane is the rupture of the fetal membranes before the onset of labour. In most

cases, this occurs near term, but when membrane rupture can occurs before 37 weeks' gestation, it is known as preterm premature rupture of membranes (PPROM).³

Prolonged rupture of membranes anytime PROM lasts longer than 18 hours before delivery, it is described as prolong rupture of membrane.⁴

The aim of this was to determine the value of aspartate aminotransferase (AST) and alanine aminotransaminase (ALT) in diagnosis of preterm premature rupture of membrane.

METHODS

This case-control study was performed at Al-Imamein Kadhimein Medical city hospital, during the period between August 2015 to May 2016 in Baghdad city, after approval by the supervising committee of Arabic Board of Obstetrics and Gynecology. One hundred pregnant women were enrolled in this study, the gestational age was between 28-36 weeks, they were divided in two groups: Group 1 (cases group): 50 pregnant women who had diagnosis of premature rupture of membranes at gestational age of 28 to 36 weeks.

Group 2 (control group): 50 pregnant women referred to obstetrics clinic for periodic check-up and had a gestational age of 28 to 36 weeks. Gestational age was determined based on the first day of the last menstrual period and first trimester ultrasound.

Exclusions criteria included: congenital malformations, fetal growth restriction, fetal distress, placenta previa, vaginal bleeding, vaginal infection and maternal disease; pregnancy hypertension, preeclampsia, and complications of pregnancy. While the inclusion criteria were; pregnant women with GA 28-36 who did not have the mention problems entered the study.

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 ultrasound for amniotic fluid index (AFI).

Method for measurement of vaginal fluid AST and ALT. In all patients, 5 cc of sterile normal saline was poured into the posterior fornix of the vagina by syringe 5 cc, and after a few minutes, the liquid was aspirated by the same syringe and was sent the laboratory of hospital and was centrifuged for 10 minutes. Alcyon automatic set and commercial kits were used to measure the concentration of aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Statistical analysis: Each patient assigned a serial identification number. The data were analysed using Statistical Package for Social Sciences (SPSS) version 20.

- Reliability tests (sensitivity, specificity, positive and negative predictive values) and critical cut-off points were calculated using receiver operator curve (ROC).

- p-value less than 0.05 was used as alpha level of significance.

RESULTS

The mean gestational age was 33.1±2.4 weeks in the case group, and 33.1±2.3 weeks in the control group, with no significant difference between them (p=0.942), there was no significant difference between mean maternal age of case group (25.5±3.8 years) and that of control group (25.6±3.2 years) (p=0.843), also no difference in parity in both group the mean for cases is 2.26±1.6 and for control group is 2.52±1.6 (p-value=0.414), also there was no statistically difference between two groups of with PPRM and without PPRM in terms of abortion and body mass index (BMI), p-value was 0.851, 0.937 respectably.

Mann-Whitney revealed that there was a statistically significant difference regarding AST, ALT measurement from vaginal washing fluid between cases and controls. The median of ALT (IU/ml) in cases and control group was 6.9 (4.5-15.2), 4.6 (3.1-7.6) respectively and p-value=0.004, and median of AST (IU/ml) of cases and control group was 8.8 (5.5-17.2), 5.1 (2.9-8.2) and p≤0.001. Also amniotic fluid index (AFI) was lower in PPRM group than in control group with median of 5 (6-7) cm, and 13.5 (15.5-16.7) cm in cases and control group respectively, Table 1.

There was a significant positive correlation between vaginal AST and ACT especially at lower values. Alanine aminotransferase (ALT) (IU/L) r-value=0.680 and p-value<0.001, as in Table 2.

		Aspartate aminotransferase (AST), IU/L
Alanine amino-transferase (ALT), IU/L	r-value	0.680
	p-value	<0.001**
** Correlation is significant at the 0.01 level (2-tailed).		

Table 2. Correlation between vaginal ALT (IU/L) and vaginal AST (IU/L) in the study groups.

Based on the results of the study, the sensitivity, specificity, positive and negative predictive values for aspartate aminotransferase (AST) were obtained 80%, 58%, 65.6%, and 74.7% respectively. And for alanine aminotransferase (ALT) were obtained 78%, 48% 60%, and 68% respectively. 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), as in Figure 1 and 2.

Variables	Cases N=50 Median (IQR)	Control N=50 Median (IQR)	Mann-Whitney U	p-value
Alanine aminotransferase (ALT) (IU/L)	6.9 (4.5-15.2)	4.6 (3.1-7.6)	-2.885	0.004*
Aspartate aminotransferase (AST) (IU/L)	8.8 (5.5-17.2)	5.1 (2.9-8.2)	-4.040	<0.001*
Amniotic fluid index (AFI) (cm)	5 (6-7)	13.5 (15.5-16.7)	-8.62	<0.001*
IQR=Inter-quartile range, *Significant at 0.05 level				

Table 1. Comparison between cases and controls as regard vaginal AST (IU/L) and ALT (IU/L) levels & U/S investigations results.

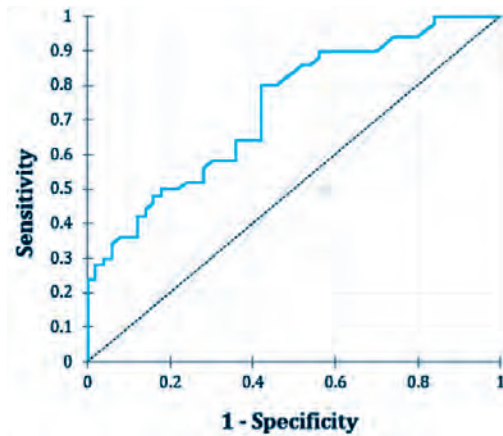


Figure 1. Receiver operator curve1 (ROC) for ALT.

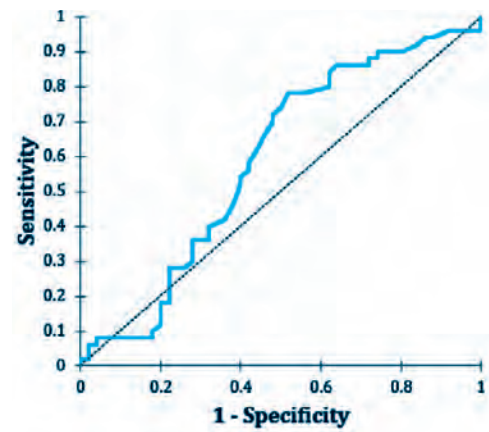


Figure 2. Receiver operator curve2 (ROC) for AST.

DISCUSSION

Premature rupture of membranes is fairly a common complication of pregnancy and can lead to increased maternal complications, operative procedures, neonatal morbidity and mortality.²

The present study was undertaken to determine the diagnostic value of AST and ALT levels in vaginal fluid for the diagnosis of preterm premature rupture of membranes as a non-invasive and available tests. AST and ALT are generated by fetus liver and secrete in amniotic fluid, and there is no relation between these amount and that of maternal enzymes.⁵ The intensity of AST and ALT in amniotic fluid increase with increased gestational age.⁵

The current study was a case-control study which was performed on 100 pregnant (50 as cases and 50 controls) women between 28-36 completed weeks of gestation to detect the vaginal levels of AST and ALT.

The study did not show significant differences in demographic distribution (in term of age, gestational age, parity, abortion, BMI) of the two groups (cases and control), vaginal fluid AST and ALT concentration was significantly higher in women with PPRM compared to women of the control group. In comparing this study to others.

The study of Kale and colleagues in 2008 was

performed on 84 women 36 pregnant women with a diagnosis of PPRM and 48 pregnant women without PPRM. The study did not show significant differences in demographic distribution, levels of ALT and AST were compared in the two groups and AST concentration in vaginal fluid was significantly higher in the group with PPRM than the group without PROM (17.46 ± 14.4 vs. 7.8 ± 3.08 IU/L, $p=0.001$), and desirable cut-off point 3, sensitivity 91%, specificity 83%, positive predictive value 80% and negative predictive value 93% were achieved. Vaginal fluid ALT concentration was slightly higher in women with PROM 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.⁵

In a study by Ali Farid et al 2011, about the levels of AST and ALT of the vaginal fluid, 45 pregnant women with PROM and 45 pregnant women without PPRM were compared, with GA 26-36th weeks, there was no statistically significant difference between cases and controls as regards demographic data (age, GA, parity abortion, BMI), AST and ALT levels were significantly higher in the group with PPRM than the group without PPRM ($p<0.001$). Cut-off point for AST was 1.25 IU/L sensitivity 97.8%, specificity 62.2% and negative predictive value of 96.55% were obtained.⁶

The cut-off point for ALT was 0.5 IU/L, sensitivity 87.7%, specificity of 62.2%, negative predictive value 85% and positive predictive value 78% were achieved.⁷

In the study of Asgharnia et al in 2014 that aimed to identify the predictive value of AST and ALT of vaginal fluid for diagnosis of PPRM, 148 pregnant women were studied with GA 26-36th weeks and the results showed no significant demographic distribution (GA, maternal age) between PROM group and control group, the mean of AST level in vaginal fluid was 12.77 ± 10.06 in PPRM group vs. 6.91 ± 10.92 in control group ($p < 0.001$), optimal cut point of AST for the diagnosis of PPRM was 4.5 IU/L in this study. The sensitivity, specificity, positive and negative predictive values were 82.4%, 63.5%, 69.32% and 78.33% respectively.⁸ No significant difference between ALT levels in two groups, 1.51 ± 3.17 IU/L and 0.89 ± 1.15 IU/L respectively, $p = 0.49$.

In study of Marzieh Ghasemi et al April 2015 about diagnostic value of AST and ALT of vaginal fluid in the diagnosis of preterm premature rupture of membranes, 160 pregnant women (80 women with PROM as case group and 80 pregnant women without PROM as control group), GA 28-40th weeks were enrolled.

There was no significant difference between two groups of with PROM and without PROM in terms of demographic characteristics (age, GA, parity, gravid, abortion), levels of AST and ALT of vaginal fluid was different in the two groups. This difference was statistically significant ($p < 0.001$). Sensitivity and specificity of AST with a cut-off 7.5 IU/L were 79.7%, 56.2% respectively, positive and negative predictive values were 64.2%, 73.7% respectively. The sensitivity, specificity, positive and negative predictive values for ALT 78.4%, 48.7%, 60.1%, and 69.6% respectively, and cut off value 2.5 IU/L.⁸ In this study, the difference was observed in the two enzymes, the result may with same or slightly lower sensitivity and spasticity comparing to studies mention, this may be due to the small number of cases, small number of available studies, and the weakness of current study was possible laboratory mistakes and the use of serum kits which might not have the right accuracy for vaginal washing fluid.

In this study we use ultrasound for amniotic fluid

index (AFI) with sterile speculum and observation of cervical fluid leakage or accumulation of fluid in the posterior fornix of the vagina to confirm diagnosis of PPRM.

Ultrasound was significant in diagnosis of PPRM with $p\text{-value} \leq 0.001$, but in ultrasound should not be used as the primary means of diagnosis of PROM. False positive findings may occur in patients with oligohydramnios resulting from causes other than PROM, and false negative results may occur in patients with discrete amniotic fluid losses.

However it should be assumed that PROM has occurred if ultrasound examination shows little or no fluid in the uterus. In contrast, the presence of a normal amount of fluid makes the diagnosis of PROM unlikely.² The most reliable method for diagnosing PPRM is visualizing amniotic fluid draining through the cervix.⁷ However, certain diagnosis of PPRM is sometimes difficult, so several suggestions have been made for methods to confirm the diagnosis of PPRM when in doubt, 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 PPRM.

Studies on several markers (as mention in introduction) have been conducted including: β HCG, thyroid hormones, urea, creatinine^{6,9,10} with the exception of urea and creatinine, all of them maintained the same or lower specificity and sensitivity values comparing to our markers.^{6,9,10}

By compare the results of this study to the results of other studies marker, our markers (AST, ALT) are 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 AST and ALT of vaginal fluid can be used as convenient and fast reliable test for the diagnosis and prediction of PPRM.

CONCLUSIONS

We concluded that vaginal AST and vaginal ALT could be used as predictive test for detection of PPRM.

So, this could limit unnecessary obstetric interventions, including hospitalization, administration of antibiotics and corticosteroids, and even induction of labour and iatrogenic prematurity.

It is rapid, easy performed test as the commercial kits are available in most of hospital labs with reasonable cost.

RECOMMENDATIONS

To further investigate our hypothesis whether the association between preterm premature rupture of membrane and aspartate aminotransferase (AST) and alanine transaminase (ALT) we suggest:

1. Due to small number of studies it seems that further studies with large population should be performed.
2. Long duration time studies with more gestational age involve.
3. Further researches are required to determine the cut-off level of AST and ALT in cases of suspected leaking patients.

REFERENCES

1. Chandiramani M, Tribe RM, Shennan AH. Preterm labour and prematurity. *Obstet Gynaecol Reprod Med* 2007 Aug;17(8):232-7.
2. Patil S, Patil V. Maternal and foetal outcome in premature rupture of membranes. *Int Organ Sci Res Dent Med Sci* 2014;13(12 Ver. VII):56-83.
3. Practice bulletins No. 139: Premature rupture of membranes. *Obstet Gynecol* 2013 Oct;122(4):918-30.
4. Callahan TL, Caughey AB. Obstetrics and gynecology. *Angew Chemie Int Ed* 2013 Mar;40(6):9823.
5. Kale E, Kuyumcuoglu U, Kale A, et al. A new and practical aspartate aminotransferase test in vaginal washing fluid for the detection of preterm premature rupture of membranes. *Fetal Diagn Ther* 2008;24(4):425-8.
6. Esim E, Turan C, Unal O, et al. Diagnosis of premature rupture of membranes by identification of β -HCG in vaginal washing fluid. *Eur J Obstet Gynecol Reprod Biol* 2003 Mar;107(1):37-40.
7. Farid AA, Rabei N, AAS. Department aspartate aminotransferase and alanine aminotransferase in vaginal fluid for detecting preterm premature rupture of membranes. *Int J Analyt Pharma Biom Science* 2011;7(Dec 2009):243-7.
8. Asgharnia M, Mirblouk F, Salamat F, et al. Predictive value of aspartate aminotransferase and alanine aminotransferase levels in vaginal fluid for the diagnosis of premature rupture of membranes. *Iran J Reprod Med* 2014 Apr;12(4):269-74.
9. Rashidi M, Barzegar S, Najmi Z, et al. Diagnosis of premature rupture of membranes by the detection of thyroid hormones in vaginal secretions. *Shiraz E Med J* 2015;16(11-12):10-4.
10. Kariman N, Afrakhte M, Hedayati M, et al. Diagnosis of premature rupture of membranes by assessment of urea and creatinine in vaginal washing fluid. *Iran J Reprod Med* 2013 Feb;11(2):93-100.