Measuring Aspartate aminotransferase(AST) and Alanine aminotransferase (ALT) enzymes in vaginal washing fluid for the detection of preterm premature rupture of membranes

A protocol

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Introduction

Preterm premature rupture of membranes is spontaneous rupture of the fetal membranes that occurs before 37 completed weeks and before the onset of labor.(***Mercer, 2003)".*** Preterm premature rupture of membranes occurs in approximately 3% of all pregnancies and it is associated with 30-40% of preterm deliveries.(**Marowitz, Jordan, 2007).**

At term,programmed cell death and activation of catabolic enzymes, such as collagenase and mechanical forces, result in ruptured membranes.preterm PROM occurs probably due to the same mechanisms and premature activation of these pathways.However,preterm PROM also appears to be linked to underlying pathologic processes, most likely due to inflammation and/or infection of the membranes. Clinical factors associated with preterm PROM include low socioeconomic status, low body mass index, tobacco use, preterm labor history, increased intrauterine pressure as multifetal pregnancy and polyhydraminos, urinary tract infection, vaginal bleeding at any time in pregnancy, cerclage, and amniocentesis.(**Allahyar ,2008)**.

One of the most common complication of preterm premature rupture of the membranes is preterm delivery within week (Rate of incidence is 50-75%), respiratory distress syndrome (35%), cord compression (32-67%),chorioamnionitis(13-60%), abruptio placentae(4-12%);antepartum fetal death(1-2%).(**Tany , Ashley ,2006).**

The diagnosis of prterm PROM is made by history and physical finding consisting of demonstration of visible amniotic fluid in the vaginal fornices and performing fern and nitrazine tests. Unfortunately, nitrazine paper testing of vaginal PH has a false –positive rate associated with blood contamination, semen, or bacterial vaginosis. Fern test also has a false-positive rate. Diagnosis of PPROM is not supported by vaginal pooling of amniotic fluid when it is slight in amount. (**Carroll ,Blott et al.,2000)**

These potential limitations have led to the search for the secretory biochemical markers for the detection of PPROM. Among the markers evaluated were ß-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 , Turan et al.,2003).**

The most recent method is to measure aspartate aminotransferase(AST) and alanine aminotransferase(ALT) in vaginal washing fluid for the detection of preterm PROM.(**Ebru , Umur et al.,2008).**

Liver enzymes of aspartate aminotransferase (Ast) and alanine aminotransferase (ALT) are produced by the fetus. These levels do not correlate with maternal levels Concentrations of Ast and Alt all increase with gestational,age**.** Their concentrations in the amniotic fluid have been shown by different studies. (**Kuczynska ,Wojcicka et al., 1989)(Smolarczyk ,Wojcicka et al., 1996).**

Alanine transaminase or ALT is a transaminase enzyme. It is also called serum glutamic pyruvic transaminase (SGPT) or alanine aminotransferase(ALAT).
ALT is found in serum and in various bodily tissuses.It catalyzes the transfer of an amino group from alanine to a-ketoglutarate,the products of this reversible transamination reaction being pyruvate and glutamate.
It is commonly measured clinical as a part of a diagnostic liver function test.It is almost always measured in units/liter (U/L).(**Paul ,2005).**

Aspartate transaminase or **AST**also called serum glutamic oxaloacetic transaminase (SGOT) or aspartate aminotransferase(ASAT/AAT) is similar to alanine transaminase in that it is another enzyme associated with liver parenchymal cells.
It facilitates the convertion of aspartate and alpha-ketogluterate to oxalacetate and glutamate,and vice-versa.It is measured in units/liter.(**Gaze ,2007)**.

Aim of the Work

The aim of this study is to measure aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes levels in vaginal washing fluid in order to detect preterm premature rupture of membranes.

Patients and Methods

This study will be conducted at Maternity Hospital of Ain Shams University on 74 pregnant women[calculated by using Epicalc program 2000 for detection of the sample size for case control study using mean values at power 80%, confidence interval 95% ,(significant P at ≤ 0.05)] who are admitted to the hospital with the diagnosis of preterm PROM (n=37study group) and normal pregnancy(n=37 control group).

Inclusion criteria:-

* Gestational age is enrolled between 26 and 36 completed weeks of pregnancy

Exclusion criteria:-

- No fetal congenital anomalies.

- No fetal growth restriction.

- No fetal distress.

- No placenta previa.

- No vaginal bleeding.

- No pregnancy-induced hypertension or pre-eclampsia.

As all these conditions have an impact on vaginal AST and ALT hormone concenteration.

Upon admission:-

1- History taking considering a history of leakage of vaginal fluid suspected to be amniotic fluid and first date of the last normal mensterual period to estimate the gestational age.

2- Routine laboratory investigations e.g CBC, ABO, Rh typing ,…,….

3-Fetal heart rate non-stress test.

4- Trans-abdominal ultrasound to estimate the gestational age, to exclude congenital anomalies, placenta previa, and fetal growth restriction

5- A sterile speculum examination to confirm fluid leakage from the cervical canal or pooling of fluid in the posterior vaginal fornix.

6- PH measurement of vaginal fluid will be done using nitrazine test paper.
All patients will be classified into two groups: control group and study group.

The study group:-

In the patients of this group, the vaginal fornices will be irrigated with 3 ml of sterile saline and a 5- ml syringe; with the same syringe, fluid was aspirated from the posterior vaginal fornix within 6 hour after the onset of leakage of vaginal fluid. The fluid specimens collected to the polypropylene tubes will be immediately centrifuged for 10 min at 3,500ġ and the supernatant fluid will be stored at -80ºC until laboratory analysis. For the measurement of AST and ALT concentration, a photometric method will be applied using commercial kits(Abbott, Abbott park, ILL., USA) with the aeroset analyzer(Abbott, Japan),(Ellen ,George ,et al.,2001).

The control group:-

After confirming with negative nitrazine and fern testes, and the absence of the amniotic fluid pooling in the posterior fornix for the control group, The sampling method of AST and ALT assays will be as previously described for the study group.
The results of the two groups will be compared according to the standard statistical methods .

Results.

Data will be expressed as mean ± SD.the receiver operator characteristic (ROC)curve analysis will be used to establish the optimal cutoff values for vaginal fluid AST and ALT concentrations in the prediction of PPROM. Sensitivity, specificity, positive, and negative predictive values of the AST test will be calculated.

Discussion.

Conclusion.

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قياس خمائر الاسبارتات ترانس أمينيز والألانين ترانس أمينيز فى السائل المهبلى لتشخيص الإنفجار المبكر لجيب المياه الامنيونى

إطار بحث

مقدم للحصول على درجة الماجستير فى أمراض النساء والتوليد

إعداد

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الملخص العربي

المقدمة

إن الانفجار المبكر لجيب المياه الأمنيوني يعرف بأنه انفجار هذا الجيب قبل بدء الولادة وقد وجد انه يحدث في٣% من الحوامل ويصاحبه ولادة مبكرة بنسبة ٣٠-٤٠ % من الولادات المبكرة ٠

والسبب المباشر لإنفجار جيب المياه الأمنيونى غير معروف ولكن هناك عدةعوامل تساعد على حدوثه مثل الشيخوخة والموت المبكر للخلايا وذلك نتيجة تنشيط الأنزيمات الهادمة مثل إنزيم كولآجيناز ٠

وقد وجد أن الإنفجار المبكر لجيب المياه الأمنيونى يرتبط بعدةعوامل ومنها :-

- إلتهاب جيب المياه وإصابته بالميكروبات .
- إنخفاض الوضع الإجتماعى الإقتصادى للسيدة الحامل.
- تدخين السيدة الحامل وتاريخ سابق لولادة مبكرة فى الماضى.
- زيادة الضغط داخل الرحم فى حالات تعدد الأجنة(الحمل التوأمى) وزيادة السائل الامنيونى.
- ربط عنق الرحم.
- بزل السائل الأمنيونى.
- إلتهاب عنق الرحم.

وهذا الإنفجارالمبكر لجيب المياه الأمنيونى يؤدى إلى العديد من المضاعفات التى تحدث للجنين والأم ومنها دخول الأم فى ولادة مبكرة والضغط على عنق الرحم وإلتهاب الغشاء المشيمى الأمنيونى المحيط بالجنين والإنفصال المفاجئ للمشيمة وموت الجنين قبل الولادة٠

ويتم تشخيص الإنفجار المبكر لجيب المياه الأمنيونى عن طريق أخذ التاريخ المرضى من السيدة الحامل والفحص بالمنظار المهبلى لرؤية هذا السائل الأمنيونى بالمهبل وعمل عدة إختبارات ومنها قياس الرقم الهيدروجينى فى السائل المهبلى والذى يتميز بإرتفاع تركيزه ولكن من الممكن أن يعطى نتائج خاطئه وذلك لوجود دم أو سائل منوى أوإلتهاب ميكروبى بالمهبل.

ونتيجة لضعف هذا الإختبار فى تشخيص الإنفجار المبكر لجيب المياه الأمنيونى أخذ فى البحث عدة إختبارات أخرى ومنها قياس هرمون البرولاكتين وهرمون الكالسى تروبك بالسائل المهبلى ٠

ومن أحدث هذه الإختبارات لتشخيص الإنفجار المبكر لجيب المياه الأمنيونى هو قياس خمائر(إنزيمات) الترانس امينيزأسبارتات – الألانين فى السائل المهبلى٠

حيث أن خمائر(إنزيمات) الترانس أمينيز (الأسبارتات ترانس أمينيز- والألانين ترانس أمينيز) تنتج عن طريق الجنين وتركيزها غير مرتبط بتركيز هذه الخمائر(الانزيمات) الخاصة بالأم ٠

الهدف من الدراسة :-

الهدف من الدراسة هو معرفة تركيزمحدد ومميز لمستوى خمائر(إنزيمات) الترانس أمينيز (أسبارتات-الألانين)فى السائل المهبلى وذلك لتشخيص إنفجار جيب المياه المحيط بالجنين

المرضي وطرق البحث :-

سوف يتم إجراء هذه الدراسة في مستشفى أمراض النساء والتوليد التابعة لكلية الطب جامعة عين شمس على ٧٤ سيدة حامل جئن إلى عيادة النساءوالتوليد ومنهن ٣٧سيدة حامل يشكين فقط من نزول سائل من المهبل وهؤلاء المريضات يتميزن بـ:-

١ – مدة الحمل من ٢٦ حتى ٣٦ أسبوع.
٢ – لا يوجد عيوب خلقية بالجنين.
٣ – لا يوجد قصور بالنمو الجنينى.
٤ – لا يوجد الإندغام المعيب للمشيمة.
٥ – لا يوجد نزيف مهبلى.
٦- لا يوجد إرتفاع فى ضغط الدم مع الحمل.
حيث أن هذه الحالات لها تأثير على تركيز خمائر(إنزيمات) الترانس أمينيز (الأسبارتات – الألانين) فى السائل المهبلى وعند الدخول للمستشفى يتم أخذ التاريخ المرضى ثم فحص الحالات بواسطة المنظار المهبلى وعمل إختبار للجنين لمعرفة حالتة الصحية وعمل فحص بالموجات فوق الصوتية على البطن والحوض وقياس الرقم الهيدروجينى للسائل المهبلى بإستخدام ورق عباد الشمس.

سوف تقسم الحالات إلى مجموعتين :-

١- المجموعة الأولى :
٣٧سيده حامل تعاني من انفجار مبكر لجيب المياه وبها سائل امنيوني مرئي أثناء الفحص بالمنظار المهبلي ونتيجة إجابية لورقة عباد الشمس.

٢ – المجموعة الثانية :
٣٧ سيده طبيعية أثناء الفحص بالمنظار المهبلي ونتيجة سلبية لورقة عباد الشمس.

سوف يتم التالى لكلا المجموعتين وهو حقن ٣ ملى من محلول معقم فى المهبل وسحب ٥ ملى من السائل الموجود بالمهبل خلال ٦ ساعات من حدوث الإنفجار المبكر لجيب المياه الأمنيونى أو عدمه ويتم فحص هذا السائل وتجميده لحين تحليله وسوف يتم مقارنة النتائج الخاصة بالمجموعتين بناء على القواعد الإحصائية المعروفة.

Introduction

Preterm prelabor rupture of membranes refers to rupture of the fetal membranes before the onset of labor at less than 37 weeks gestation **(Geun and Myoung, 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 the fetal membranes followed by a sterile speculum examination demonstrating pooling of fluid in the posterior vaginal fornix; ultrasound 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 changes. 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 **(Cunningham 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; Ebru et al., 2008).**

Amniotic fluid in the second half of human gestation is largely a product of fetal urine and additional sources of amniotic fluid are respiratory and gastrointestinal tract excretions **(Muller al, 1994; Fauza, 2004)**.

Evidence suggests that liver enzymes of aspartate amino transferase (AST) and alannine amino transferase (ALT) are produced by the fetus. These levels do not correlate with maternal levels **(Ebru et al., 2008)** and their concentrations in the amniotic fluid have been shown by different studies **(Kuszynska-Sicinska et al., 1989; Smolarczyk et al., 1996; Ebru et al., 2008)**.

There is no available information about AST and ALT levels in vaginal washing fluid until now. Therefore, this study will measure and compare AST and ALT levels in the vaginal washing fluid of normal pregnant women and pregnant women with PPROM in a trial to confirm the results of a previous study in that issue **(Ebru et al., 2008)**.

Aim of the Work

The aim of this study is to measure aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes levels in vaginal washing fluid in order to detect preterm premature rupture of membranes.

The Fetal Membranes

Fetal membranes:

The membranous structure that surrounds the developing fetus and forms the amniotic cavity is derived from fetal tissue and is composed of two layers: the amnion (inner layer) and the chorion (outer layer). The amnion is a translucent structure adjacent to the amniotic fluid, which provides necessary nutrients to the amnion cell. The chorion is a more opaque membrane that is attached to the decidua (i.e, maternal tissue that lines the uterus during pregnancy). Intact, healthy fetal membranes are required for an optimal pregnancy outcome ***(Seth, 2006).***

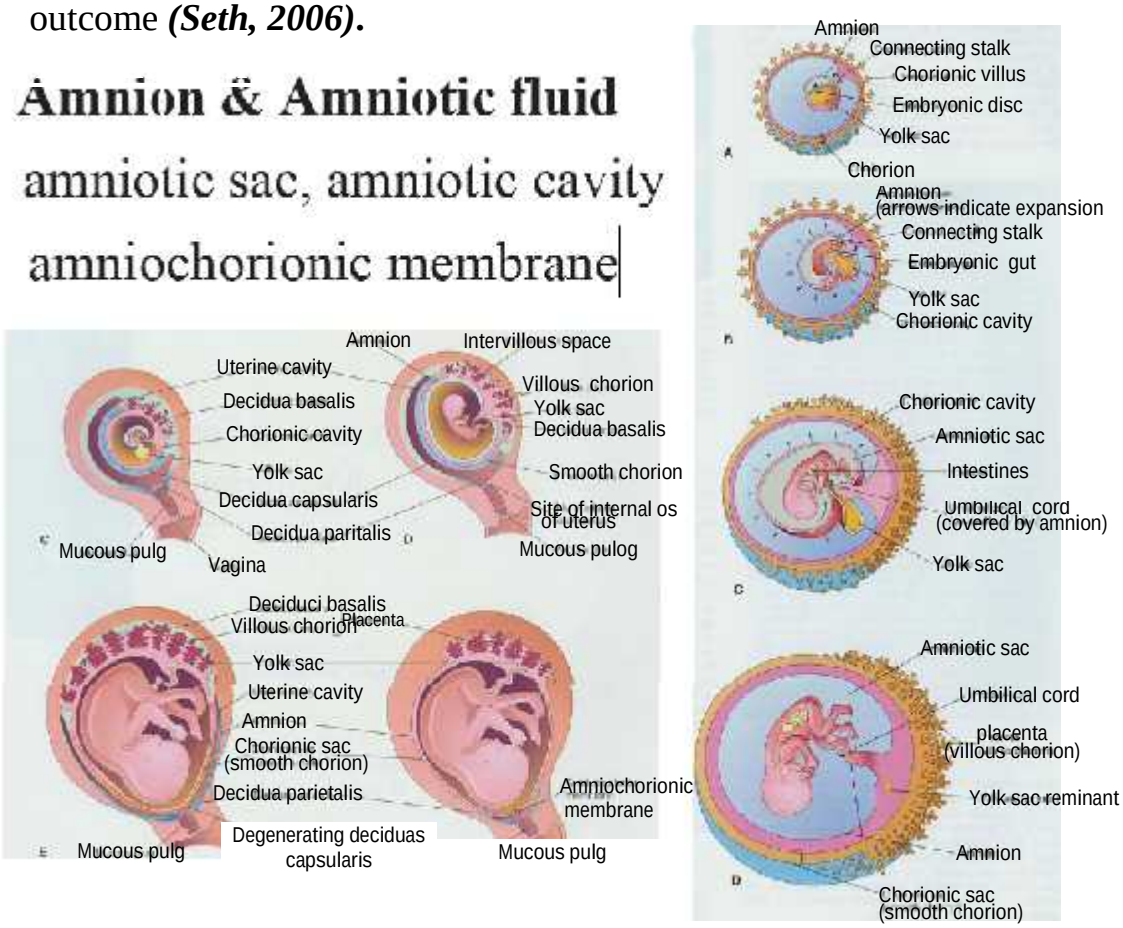

Fig. (1): Amnion and Amniotic fluid. Amniotic sac, amniotic cavity amniochorionic membrane **(Yong, 2009).**