

The Value of Free Thyroid Hormones and Aspartate Aminotransferase in Vaginal Washing Fluid for Detection of Preterm Pre-Labor Rupture of Membrane

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ABSTRACT:

BACKGROUND:

Preterm pre-labor rupture of membrane occurs in about 2% of all pregnancies. The most frequent consequences of preterm pre-labor rupture of membrane is preterm delivery, sepsis and pulmonary hypoplasia. The correct diagnosis of preterm pre-labor rupture of membrane is crucial and successful management will be based upon it.

OBJECTIVE:

To determine whether measurement of vaginal washing fluid aspartate aminotransferase (AST), free triiodothyronine (free T3) and thyroxine (free T4) were useful tests for the diagnosis of preterm pre-labor rupture of membrane.

STUDY DESIGN: A case - control study.

SETTING: This study was conducted at department of Obstetrics and Gynecology of AL-Yarmouk teaching Hospital and National center for Diabetes

PATIENTS, MATERIALS AND METHODS:

It included hundred pregnant women with a gestational age ranging between 24 - 36+6 weeks. They were divided into two groups, the study group which included fifty pregnant women presented with preterm pre-labor rupture of membrane and the control group which includes fifty pregnant women without any complaint, matched for gestational age. All women underwent sterile speculum vaginal examination. Free triiodothyronine and free thyroxine levels were measured by using Enzyme immune assay method, and aspartate aminotransferase level was measured by using colorimetric method, in vaginal washing fluid.

RESULTS:

Vaginal washing fluid free T3, free T4 and AST levels were significantly higher in the study group (1.15), (0.071), (4.9) respectively compared with the control group (0.93), (0.048), (4.5) respectively. Vaginal washing fluid free T4 having the largest area under the curve on receiver operating characteristic curve (ROC) analysis ($P < 0.001$). ROC curve analysis showed that free T3 validity results in predicting preterm pre labor rupture of membrane among pregnant women were (cut-off 1.07pg/ml, sensitivity 88%, specificity 76% and accuracy 82%), free T4 validity results were (cut-off 0.055pg/ml, sensitivity 84%, specificity 80% and accuracy 82%) and AST validity results were (cut-off 4.9IU/L, sensitivity 52%, specificity 80% and accuracy 66%).

CONCLUSION:

Measurement of aspartate aminotransferase free triiodothyronine, and thyroxine in vaginal washing fluid of suspected and diagnosed patient presented with preterm pre labor rupture of membrane found to be a useful markers for the diagnosis of preterm pre labor rupture of membrane.

KEYWORDS: thyroid hormones, aspartate aminotransferase, pre-labor rupture of membrane.

INTRODUCTION:

Pre-labor rupture of membrane (PROM) is the

rupture of the fetal chorioamniotic membrane before the onset of labor. If rupture occurs before 37 weeks of gestation, it is called preterm pre-labor rupture of membrane (PPROM)(1).

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PROM and PPRM usually found in 10% and 40% of term and preterm labors, respectively⁽²⁾. Preterm PROM occurs in nearly 3% of all pregnancies⁽³⁾. The most frequent complications of PPRM is preterm delivery, with about 50% of pregnant delivered within a week, 75% within 2 weeks, 85% within 1 month⁽⁴⁾ and 30-40% will go on to deliver their infant at term⁽⁵⁾.

Precise and early diagnosis of PROM will lead to proper obstetric intervention and will decreased fetal and maternal complications^(6,7). On the other hand, inaccurate diagnosis may lead to unnecessary interventions; hospitalization, overuse of corticosteroids and antibiotics and even unnecessary induction of labor⁽⁸⁾. The diagnosis of PROM usually based on a history of watery vaginal loss, pooling of amniotic fluid in the vagina seen by sterile speculum examination^(9,10,11,12). Confirmation of diagnosis is confirmed by a nitrazine paper test, yet it may have false positive results because of contamination with different materials like blood, semen, urine, and vaginal inflammation. Ferning test of the cervicovaginal discharge is another classic test, but again the results are easily interrupted by the presence of semen, blood or cervical mucus⁽¹³⁾. Another important diagnostic tool is using ultrasound to assess amniotic fluid volume and in those with a strong history of PPRM but a negative speculum examination, particularly if their symptoms persist for 48 hours or more. Markers which are present in high concentrations in amniotic fluid and are therefore considered candidate for a diagnostic test include fetal fibronectin, insulin-like growth factor binding protein 1, beta-human chorionic gonadotrophin and alpha microglobulin-1 protein. These tests considered rapid with high sensitivities and specificities and commercially available. In some women rupture of membranes, fetal fibronectin testing has been shown to become negative for more than 12 hours if liquor is not seen on speculum examination⁽¹⁴⁾.

The fetus usually depends on maternal thyroid hormones until age of twelve weeks. The thyroxin level then increases until the age of 34 weeks of gestation⁽¹⁵⁾. On the other hand, Fetal triiodothyronine (T3) remains low (less than 15 ng/dL) until 30 weeks of gestation, and increases to 50 ng/dL at term. The thyroid hormones are essential for proper development and differentiation of all cells of the human body⁽¹⁶⁾.

AST (Aspartat Amino Transferase) also called serum glutamic oxaloacetic transaminase (SGOT) and ALT (Alanine Amino Transferase) formerly called serum glutamic pyruvic transaminas (SGPT) are liver enzymes that are also generated by fetus liver and secreted in amniotic fluid and there is no relation between their amount and that of maternal enzymes. The intensity of AST and ALT in amniotic fluid increases by the increased gestational age⁽¹⁷⁾. AST is similar to ALT in that it is another enzyme associated with liver parenchymal cells. But is also present in red blood cells, and cardiac and skeletal muscle, so is not specific to the liver. while AST is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas^(18,19).

AIM OF STUDY:

To determine whether the measurements of vaginal washing fluid aspartate aminotransferase, free triiodothyronine and thyroxin are useful for the diagnosis of preterm pre-labor rupture of membrane.

PATIENTS AND METHODS:

This is a case control study conducted in department of Obstetrics and Gynecology of AL-Yarmouk teaching Hospital and National center for Diabetes for the period from February 2016 through October 2016. The study protocol was approved by Scientific Council of Obstetrics and Gynecology specialization / Iraqi Board for Medical Specializations. The study included 100 pregnant women attending the outpatient clinic who were informed about the nature of the study and verbal consent was obtained from them. The women were divided into two groups: Group A (study group): includes 50 pregnant women presents with PPRM and objective visualization of amniotic fluid pooling on a sterile vaginal speculum examination who were admitted either to the obstetrical ward or to the delivery suite.

Group B (control group): includes 50 pregnant women without any complaint, matched for gestational age. The inclusion criteria was Single viable pregnancy, gestational age ranging between 24 to 36+6 weeks confirmed by obstetrical criteria depending on accurate last menstrual period and / or by early ultrasonography. The exclusion criteria includes :multiple pregnancies, regular uterine contraction, vaginal bleeding, fetal compromise or with known fetal malformation, chorioamnionitis, Abnormal placentation and a history of vaginal douching or sexual intercourse in the previous 24 hours. Any preexisting medical disease including thyroid, liver, renal or hypertensive disease also was excluded.

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Detailed history were obtained including age, parity, gravidity, history of abortion, gestational age, obstetrical history, gynecological history and past medical history and general examination, systemic examination, and vital signs were recorded.

Obstetrical examination includes the following: Abdominal examination for assessment of symphysis fundal height, uterine contraction and fetal heart rate.

A sterile vaginal speculum examination for assessment of cervical state (dilatation and effacement), and pooling of amniotic fluid in the vagina. Ultrasound was done and maternal blood sample collected at the time of admission (before they were receiving antibiotics, corticosteroids and tocolysis) and sent to the laboratory for blood group, full blood count and random blood sugar.

The vaginal washing fluid sampling for (Free T3, T4 and AST) was done as follows: All women underwent a sterile vaginal speculum examination in lithotomy position. 5 ml of saline solution used to irrigate the vagina and then aspirated 3 ml of it after 3 minute, using the same syringe. The sample collected in properly labeled polypropylene tube and immediately centrifuged for 15 minute. Then the sample either stored in

freezing -4 centigrade degree or tested immediately. Free T3 and T4 estimated by Mini VIDAS which is automated quantitative enzyme linked fluorescent assay technique.

STATISTICAL ANALYSIS

The data analyzed using Statistical Package for Social Sciences (SPSS) version 21. The data presented as mean and standard deviation or median and inter-quartile range, according to their distribution, independent t- test (two tailed) or Mann-Whitney u test was used to compare the continuous variables between study groups, according to their distribution, receiver operator curve (roc) was used to assess the sensitivity and specificity of vaginal fluid free T3, free T4 and AST in the prediction of preterm pre-labor rupture of membranes.

Spearman's correlation between time interval of delivery (hours) and vaginal fluid free T3, free T4 and AST. The level of p-value less than 0.05 was considered significant.

RESULTS:

The current study involved 100 women, 50 with group A (study group) and a similar number of patients with group B (control group).

as shown in table 1, no significant differences were observed in mean age and parity between group (A) and group (B) ($p > 0.05$). a highly significant association was observed between lower gestational age mean and pregnant women with group (A) ($p < 0.001$).

Table 1: Demographic data for both group a and group B.

Variables	Group(A) (N=50)	Group(B) (N=50)	p-value
Age (years), Mean \pm SD	26.6 \pm 7.5	26.7 \pm 7.3	0.914
Parity, Median (IQR)	1 (0 - 4)	2 (1 - 4)	0.413
Gestational age (weeks) at admission Mean \pm SD	29.6 \pm 3.3	32.7 \pm 3.7	<0.001*

SD= Standard deviation, IQR= inter-quartile range, *Significant at 0.05 level

As shown in table 2, there was a significantly higher median levels of free T3, free T4 and AST among

pregnant women with group (A) than group (B) ($p < 0.05$).

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Table 2 : Comparison of vaginal fluid free T3, free T4 and AST levels between group (A) and group (B).

Variables	Group(A) (N=50) Median (IQR)	Group(B) (N=50) Median (IQR)	p-value
Free T3 (pg/ml)	1.15 (1.1-1.2)	0.93 (0.895-1.035)	0.001*
Free T4 (ng/dl)	0.071 (0.066-0.077)	0.048 (0.046-0.052)	0.002*
AST(IU/L)	4.9 (4.25-5.0)	4.5 (4.1-4.8)	0.034*

IQR= inter-quartile range, *Significant at 0.05 level

Vaginal fluid free T4 had the most significant association. Diagnostic accuracy of vaginal fluid free T3 (cut-off 1.07, pg/mL), free T4 (cut-off 0.055, ng/dL) and AST (cut off,4.9IU/L) levels

in PPRM .Vaginal fluid free T3 was the most sensitive (88%), while vaginal fluid free T4 was the most specific (80%). Vaginal fluid AST had lower diagnostic accuracy compared with free T3 and free T4 as shown in table 3.

Table 3: Diagnostic accuracy for markers of group (A).

Variables	Cut-off value	Sensitivity	Specificit-y	PPV	NPV	LR+	LR-	Accu-racy
Free T3	1.07(pg/dl)	88	76	78.6	86.4	3.7	0.16	82
Free T4	0.055(ng/dl)	84	80	80.8	83.3	4.2	0.2	82
AST	4.9(IU/L)	52	80	72.2	62.5	2.6	0.6	66

Figure 1 illustrates ROC curve analysis was constructed for vaginal fluid free T3, free T 4 and AST as diagnostic of PPRM. All of them had large significant area under the curve, indicating significant association between higher levels and diagnosis of PPRM.

Figure 1: Receiver operating characteristic curves for vaginal fluid free T3, free T4 and aspartate

aminotransferase (AST) as markers of preterm pre-labor rupture of membranes.

Spearman correlation test showed a significant negative correlation between AST level of pregnant women and time interval of delivery (p=0.03). No significant correlation was observed between free T3 andT4 level of pregnant women with time interval of delivery. All these findings were shown in table 4.

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Table 4: Correlation between time interval of delivery (hours) and vaginal fluid free T3, freeT4 and AST.

Markers	Time interval of delivery (hours)	
	r-value	p-value
FT3	-0.096	0.649
FT4	-0.325	0.113
AST	-0.434	0.03*

r is the regression coefficient, significant at 0.05 level by Spearman's correlation

As shown in table 5 , no significant correlation was observed between each of free T3 and AST

levels of pregnant women with gestational age ($p > 0.05$), just in free T4 it was highly significant observation ($p = 0.001$).

Table 5: Comparison of Laboratory marker according to gestational age at delivery in group (A).

Parameters	Early preterm birth (<=28 wks.) N=22 Mean±SD	Moderately preterm birth (29–33 wks.) N=18 Mean±SD	Late preterm birth (34–36 wks.) N=10 Mean±SD	p-value
FT3	1.12 ± 0.09	1.1 ± 0.14	1.19 ± 0.07	0.076
FT4	0.07 ± 0.01	0.06 ± 0.02	0.08 ± 0.01	0.001*
AST	4.68 ± 0.44	4.78 ± 0.47	4.76 ± 0.16	0.746

SD=Standard deviation, *Significant at 0.05 level by ANOVA

DISCUSSION:

Many complication can occur for women with PPRM including infections and preterm (before 37 weeks) babies who on the other hand can suffer from complications of prematurity, including death⁽²⁰⁾.

The current study aimed to evaluate diagnostic value of vaginal washing fluid (AST), (T3) and (T4) in women with PPRM. It showed that vaginal washing fluid of free T3 and free T4 are found to be of clinical significance in excluding many cases of wrongly suspected PPRM, thus

decrease unnecessary hospitalization, antibiotics and steroids administration.

Ali Farid et al. found that vaginal washing fluid of AST and ALT were highly significant in cases when compared to the controls p-value ($p < 0.001$) which is in agreement to the result of the current study regarding AST p-value ($p 0.034$)⁽²¹⁾

The present study also agrees with another study done by Ashgharnia et al. They found that the measurement of AST level in the vaginal washing fluid can be used as a reliable test for diagnosis of

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PPROM, p-value (<0.001), while there were no significant difference between ALT levels in PPRM group and control group, also it reported that the optimal cut point of AST for the diagnosis of PPRM was 4.5 IU/L and the sensitivity, specificity, positive and negative predictive values were 82.4%, 63.5%, 69.32% and 78.33% respectively, while in the current study the results of AST vaginal washing fluid were (cut off value 4.9 IU/L , sensitivity 52%, specificity 80%, PPV 72.2% and NPV 62.5%).⁽²²⁾

Another similar study done by Kale E. et al, found that the vaginal washing fluid ALT concentration was slightly higher in women with PPRM compared to women of the control group (p-0.064); yet, this difference did not reach statistically significance, while vaginal washing fluid AST was significantly higher in women with PPRM p-value (0.001).⁽¹⁷⁾

The above mentioned study agreed with current study in regard to AST which was statistically significant p-value (0.034), so Kale E. et al. considered AST as an excellent predictive test for detection of PPRM, but this result disagree regarding AST as an excellent predictive test when comparing vaginal washing fluid of AST with vaginal washing fluid of free T3 and free T4 for detection of PPRM in the current study p-value (p-0.001) , (p-0.002) respectively.⁽¹⁷⁾

A similar result to the current study found by Mandana Rashidi⁽²³⁾ which was carried to evaluate the values of free thyroid hormones in the vaginal washing fluid in cases of (PROM). They found that the vaginal washing fluid of free T4 values in the PROM group were significantly higher compared to the control group (p <0.0001) exactly as in the current study vaginal washing fluid of free T4 value in PPRM group were highly significant compared to the control group p-value (0.002).

The study of Kale A. and colleagues (24), concluded that vaginal washing fluid of total T3 and free T3 levels were not statistically significant in the PPRM group when compared with the control group (p-0.087) and (p-0.123) respectively , while vaginal washing fluid of total T4 and free T4 levels were significantly higher in the PPRM group when compared with the control group (p-0.002) and (p-0.000) respectively , these results were disagree with the current study regarding vaginal washing fluid of

free T3 that provide the best cut-off (0.079n/dl) , sensitivity (90%) , specificity (70%) , PPV (75.0%) and NPV (87.5%).

Another study done by Farag and colleagues⁽²⁵⁾, they found that vaginal washing fluid of free T3 and free T4 being useful and simple markers in the diagnosis of PPRM. Their results showed that sensitivity, specificity, and positive and negative predictive values for free T3 were 88%, 70%, 74.6%, 85.4%, respectively and cut off value 1.06 pg/mL, and those for free T4 were 86%, 72%, 75.4%, 83.7%, respectively and cut off value 0.063 ng/dL. This is in agreement with the current study which showed vaginal washing fluid for free T3 in predicting PPRM among pregnant women were (cut-off 1.07, pg/mL , sensitivity 88%, specificity 76% , NPV 86.4 , PPV 78.6) and vaginal washing fluid for free T4 were (cut-off 0.055 ng/dL, sensitivity 84%, specificity 80%, NPV 83.3, PPV 80.8).

CONCLUSION:

Measurement of aspartate aminotransferase (AST), free triiodothyronine(T3) , and thyroxin (T4) in vaginal washing fluid of suspected and diagnosed patient presented with preterm pre-labor rupture of membrane found to be a useful markers for the diagnosis of preterm pre-labor rupture of membrane.

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