

Vaginal lactate as a test to diagnose premature rupture of membranes

Dr.Enas M Yaseen* and Dr. Thanaa Mahmood**

*Assisted prof.in obstetrics and gynecology

College of medicine-Tikrit University

**Specialist in obstetrics and gynecology

College of medicine-Tikrit University

Abstract

Background: Premature rupture of fetal membrane is one of the most common and controversial problem facing the obstetric clinician. Its Correct diagnosis has great importance because failure of diagnosis can lead to unwanted obstetric complications or over diagnosis can lead to unnecessary intervention. **Objective:** To evaluate the reliability of vaginal lactate level as an indicator of the onset of labor in pregnant women with premature rupture of membranes. **Setting:** Tikrit Teaching Hospital, Obstetrics and Gynaecology Department, over a period of twelve months: starting from the January 2013 to the December 2013. **Study design:** A prospective observational study . This study includes eighty pregnant women between twenty eight to forty weeks of gestation. The sample size was divided into forty pregnant women with confirmed premature rupture of membrane (group 1) and forty pregnant women with suspected premature rupture of membranes(group 2). The patients underwent a sterile speculum examination and two samples of vaginal fluid were aspirated One sample was used for lactate determination and the other sample for the nitrazine paper test. Then, they were followed till their labor. **Results:** With lactate levels>5.0 mmol/l, 95.1 % of pregnant women had onset of spontaneous labor within 48 h, while within those with lactate levels<5.0 mmol/l, only 20.5 % of pregnant women had started labor within 48 h. **Conclusion:** A lactate level>5.0 mmol/l is significantly associated with spontaneous onset of labor within . 48 hrs. in pregnant with PROM.

Introduction:

Premature rupture of membrane (PROM) is the rupture of the fetal membranes with the leakage of amniotic fluid before the onset of labor (i.e. in the absence of uterine activity. When this occurs before 37 weeks gestation it is called preterm premature rupture of membranes (PPROM). Premature rupture of membrane is a common obstetrical problem, and the assessment of the women with possible membrane rupture is management issue faced in every day practice. When PROM occurs, the fetus loses the relative isolation and protection in amniotic cavity. ⁽¹⁾ It is associated with significant maternal and neonatal risks and management strategies that are often diverse and controversial. ⁽²⁾

It is generally accepted that PROM occurs in 10% of all pregnancies, with majority of cases occurring after 37 weeks gestation . PPRM occurs in approximately 3-5% of all pregnancies and accounts for one-third of all preterm births. ⁽³⁾

There is no single known cause of premature rupture of membranes. Though it is possible for the condition to occur for unknown reasons, certain risk factors have been identified, these include: ⁽⁴⁾ **1-Infection** local infection seems to be responsible initially in weakening the membranes . In fact, carriers of one or more of these sexually transmitted microorganisms (Gonorrhoea , Group B Streptococcus , Chlamydia , Trichomonas) have an increased risk of PPRM . ⁽⁴⁾ **2. Cervical incompetence:** A

weak cervix can predispose to early membrane rupture. It will fail as barrier to ascending infection and by allowing membrane to prolapse. ⁽¹⁾ **3.Vaginal bleeding:** especially if bleeding occurs later in pregnancy. Subclinical bleeding is also associated with PPRM, and may reflect decidual dysfunction. ^(2,3,5)

4. Membrane Stretch And PROM: Uterine over distention due to both polyhydramnios and multifetal gestation induces membrane stretch . Mechanical

stretching of the fetal membranes up regulates the production of several amniotic factors, including prostaglandin E2 and interleukin 8. Stretch also increases matrix metalloproteinase MMP-1 activity within the membranes. Prostaglandin E2 increases uterine irritability, decreases synthesis of fetal membrane collagen , and increases the production of MMP-1 and MMP-3 by human fibroblasts. Interleukin-8, which is produced by amniotic and chorionic cells, is chemotactic for neutrophils and stimulate collagenase activity. ⁽⁶⁾

5-Ehlers-Danlos Syndrome: A group of at least 11 heritable disorders of connective tissue characterized by hyper-elasticity of the skin and joints is caused by various defects in the synthesis or structure of collagen. ⁽⁷⁾

6-previous history: This risk is greatest for woman who had PPRM in their previous pregnancy who will have 21-32% recurrence risk in subsequent pregnancies. ⁽²⁾ **7-Nutrition:** Nutritional deficiencies that predispose to abnormal collagen structure. ⁽⁸⁾ Collagen cross — links, which are formed in a series of reactions

initiated by lysyl oxidase, increase the tensile strength of the collagens. Lysyl oxidase is a copper dependent enzyme, and women with preterm PROM have lower copper concentrations in maternal and umbilical cord serum.⁽⁹⁾ Similarly women with low serum concentration of ascorbic acid, which is required for the formation of the triple helical structure of collagen, have a higher rate of preterm PROM.⁽¹⁰⁾

7- Smoking: Tobacco smoking, which independently increases the risk of preterm PROM, has been associated with decreased serum concentration of ascorbic acid. In addition, the cadmium in tobacco has been found to increase the metal — binding protein metallothionein in trophoblasts, which may result in sequestration of copper.⁽¹¹⁾

8- Racial factor: There is an overrepresentation in the occurrence and recurrence of preterm PROM in black mothers.⁽¹²⁾

Diagnosis: To know for sure if a woman has experienced PROM, a health care clinician must prove that (1) the fluid leaking from the vagina is amniotic fluid, and (2) that labor has not yet started by:

History: A person with PROM typically recalls a sudden gush of fluid loss from the vagina, or steady loss of small amounts of fluid.⁽¹²⁾

Examination: Diagnosis should be confirmed by sterile speculum examination of the cervix, performed after the patient has rested supine for 20-30 minutes. Physician should perform a speculum examination to evaluate if any cervical dilation and effacement are present. When preterm PROM is suspected it is important to avoid performing a digital cervical examination; such examinations have been shown to increase fetal morbidity

and mortality.⁽¹³⁾ Confirmation of the diagnosis by speculum examination includes the identification of a pool of amniotic fluid in the posterior fornix (pooling).

Investigations : A- basic bedside test:

Nitrazine test: The normal vaginal pH is between 4.5 and 6.0, whereas amniotic fluid is more alkaline, with a pH of 7.1 to 7.3. Nitrazine paper will turn blue when the pH is above 6.0; however, the presence of contaminating substances (e.g., blood, semen, alkaline antiseptics) also can cause nitrazine paper to turn blue, giving a false-positive result.⁽¹⁴⁾ The Nitrazine test produces 12.7% false negative and 16.2% false positive results and its sensitivity is 77%.⁽¹³⁾

Fern test: It results from the drying out of salts contained in the amniotic fluid; the sample of fluid is placed on a glass slide and allowed to dry. The preparation is observed under the microscope looking for a crystallization pattern that resembles a fern.⁽¹⁵⁾ The fern test gives 4.8% false negative and 4.4% false positive results. The sensitivity of this test is about 62%.⁽¹⁶⁾

B-specialized test : Alpha fetoprotein test (AFP): AFP is present in high concentration in the amniotic fluid, but does not exist in vaginal secretion or in the urine; therefore determination of this substance in the vaginal secretion is an accurate test for the diagnosis of PROM with sensitivity 98% and specificity of 100%. The test may be unreliable at term because amniotic fluid AFP decreases with gestational age.⁽¹⁷⁾

Fetal fibronectin test: Is a large plasma glycoprotein, three sub-type are available of which one is fetal derived. The concentration of Fetal fibronectin in AF is 5-10 times higher than in maternal plasma.

The production of fetal fibronectin by human amniotic cells is stimulated by inflammatory mediators (including interleukin-1 and tumor necrosis factor alpha) that are considered important in initiating preterm labour. It is expensive test.⁽¹⁸⁾

Insulin like growth factor binding protein 1: Is a placental protein and it is present in high concentration in AF compared with serum and cervical mucus. It exhibit sensitivity of 81%- 100% and specificity of 71% -95% for the diagnosis of PROM. It had a rapid bedside test but it is expensive.⁽¹⁹⁾
B-HCG in vaginal washing fluid: It is a glycoprotein hormone present in high concentration in the amniotic fluid and maternal serum during

pregnancy also it is present in urine and secreted by cervical glands, therefore it is present at certain level in vaginal fluid. Several studies have documented low and stable BHCG level in the vaginal washings of pregnant women with intact membrane, where as they documented approximately nine-fold increased levels of B-HCG in the vaginal washing fluid of pregnant women with PROM.⁽²⁰⁾
Placental alpha-microglobulin-1 (PAMG-1) : PAMG-1 has undergone recent evaluation for diagnostic testing in PPROM. This 34kDa placental glycoprotein is abundant in amniotic fluid (2000-25 000 ng/mL), with much lower concentrations in maternal blood (5-25 ng/mL).²⁰ The protein is present in negligible amounts in cervicovaginal secretions with intact membranes (0.05-0.2 ng/mL).⁽²¹⁾ The 1000- to 10 000-fold difference in concentration between amniotic fluid and cervicovaginal secretions stimulated interest in a PAMG-1 immunoassay. Marketed as AmniSure (AmniSure

International, Cambridge, MA), the assay's minimum detection threshold for PAMG-1 is 5 ng/mL, sufficient for 99% accuracy with minimal false negatives. It proved superior to conventional combined clinical tests involving visualization of fluid pooling in the posterior fornix, arborization, and nitrazine testing, but it an expensive test.⁽²²⁾

Diamine oxidase test: It is an enzyme produced by the decidua which diffuses into the amniotic fluid.⁽²⁰⁾

It is absent from urine or vaginal secretion but present in large amount in amniotic fluid. Measurement of diamine oxidase is an accurate way to diagnosis PROM but there is no rapid and simple test for this enzyme.⁽¹⁾

Ultrasound examination : Examination by ultrasound may be of value in the diagnosis of PROM.. However, mild reduction of amniotic fluid volume is difficult to diagnose and has many etiologies.⁽¹⁷⁾

C-Invasive test : Amniocentesis with injection of dye to confirm amniotic membrane rupture and Ultrasonography guided transabdominal instillation of indigo carmine dye (1 mL of dye in 9 mL of sterile normal saline) and observation for fluid passage transvaginally is designated an "unequivocal" diagnostic method for confirmation of membrane rupture.⁽²³⁾

Lactate Level in Amniotic Fluid: The knowledge that amniotic fluid (AF) contains high concentration of lactate has been published for the first time in the 1970's. Some publications have suggested that the source of lactate in AF is the fetus itself, mainly through urine and lung excretion. Several reports have suggested the myometrium as the most important lactate producer:⁽²⁵⁾ The

anaerobic pathway seems to be more active in the myometrium than in striated muscles. The Lactate/Pyruvate ratio, an indicator of anaerobic metabolism, is reported to be higher in the pregnant myometrium compared with other muscles . The lactate content of pregnant uterine muscle has been reported to be doubled compare with the skeletal muscle, probably reflecting a vigorous glycolytic flow when the uterus is active, the lactate concentration in amniotic fluid

is reported to be 4 - 6 times higher as compared with fetal and maternal blood. However, from the literature it is not clear from where the high AF lactate concentration is derived.⁽²⁵⁾

Patients and Methods:

This is a prospective observational study. It was conducted at the Obstetrics and Gynecology department of Tikrit Teaching Hospital over a period of 12 months: starting from the January 2013 to the December 2013. All patients had informed consent . The study sample consisted of 80 pregnant women complaining of watery vaginal discharge for (2hrs._4 days) duration. The **inclusion criteria** were a singleton pregnancy with a history of suspected PROM without uterine contractions between 28 and 40 completed weeks of gestation.

Exclusion

Presence of vaginal bleeding

Presence of regular uterine contractions.

Multiple pregnancies.

Fetal anomalies.

History of intercourse within the last 48 hours.

Uncertain last menstrual period date.

Meconium

Fever

women were divided into two groups, Group (1) with vaginal pooling of amniotic fluid (n=40) and Group (2) without vaginal pooling of amniotic fluid (n = 40), according to pooling or visibility of amniotic fluid through speculum examination .

A detailed history, general and obstetrical examination were done for each patient. The gestational age was determined depending on accurate dating of last menstrual period ,confirmed by early ultrasound. All patients sent for ultrasound examination for assessment of gestational age, measurement of amniotic fluid index, exclusion of any congenital abnormalities of the fetus and number of fetuses.

During examination all women in the study groups had undergone a sterile speculum examination, amniotic fluid pooling with or without valsava maneuver or uterine fundal pressure was noted. The nitrazine paper test was applied at the time of speculum examination, two samples of vaginal fluid were aspirated with the help of a micro-auto pipette of 50 micro liter capacity. first sample was used for nitrazine paper test and the second sample

for lactate determination . For biochemical analysis of lactate level, a commercially available Spin react Kit was used (Spin react, S.A.U.,SPIAN ,Ref ID: 1001330).

PRINCIPLE OF THE METHOD:

Lactate is oxidized by lactate oxidase (LO) to pyruvate and hydrogen peroxide (H₂O₂), which under the influence of peroxidase (POD), 4- aminophenazone (4-AP) and 4-chlorophenol form a red quinone compound:

L-Lactate + O₂ + H₂O → Pyruvate + H₂O₂
LO

2H₂O₂ + 4-AP + 4-Chlorophenol → Quinone + H₂O
POD

The intensity of the color formed is proportional to the lactate concentration in the sample. The analysis of the sample was done by the kinetic method using an auto analyser at hospital laboratories .

Patients were closely followed up for spontaneous onset of active phase of labor with careful surveillance to diagnose,:: chorioaminionitis and time interval between sampling and labor

Statistical analysis: To evaluate the predictive capabilities of lactate concentration in vaginal fluids, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) tests were calculated using 2*2 table. The significance of difference of different means was tested using independent student-t-test for difference between two means, Statistical—significance was considered whenever the P value was less than 0.05. Receiver operating characteristic curve analysis was used to establish the optimal cut-off concentration for vaginal lactate level

Results:

Descriptive analysis or characteristics of women in different groups is represented in table (1). Using independent student-t-test. There is highly significant difference between mean time of sampling and delivery (P-value <0.05) with a significant difference in Mean lactate level (P-value <0.05).

Table (1) General characteristics of women in study groups (n=80)

Characteristics of women in the study	Group(1)n=40 n=40	Group(2) n=40	p-value
Maternal age Mean± SD Range(years)	27.4± 7.1 15-40	27.2± 7. 17-43	0.5
Parity Mean± SD Range	3.4±2.5 0-12	2.5±2.1 0-8	1.8
Gestational age(weeks) at delivery Range	31.8±4.8 28-40	33.5±5.3 30-40	1.5
Time interval between sampling and Mean± SD Range	21.1±9.2 8-48hr	72.4±20.2 36-168hr	<0.001
Mean lactate level(mmol/L) (mmol/L)	7.5±2.3	5.6±2.1	0.001

Figure (1) Receiver operator characteristic (ROC) curve chosen lactate cut-off values for diagnosis of suspected PROM as (5mmol/L) with sensitivity of(94%) and specificity of (92%) p-value (<0.05).

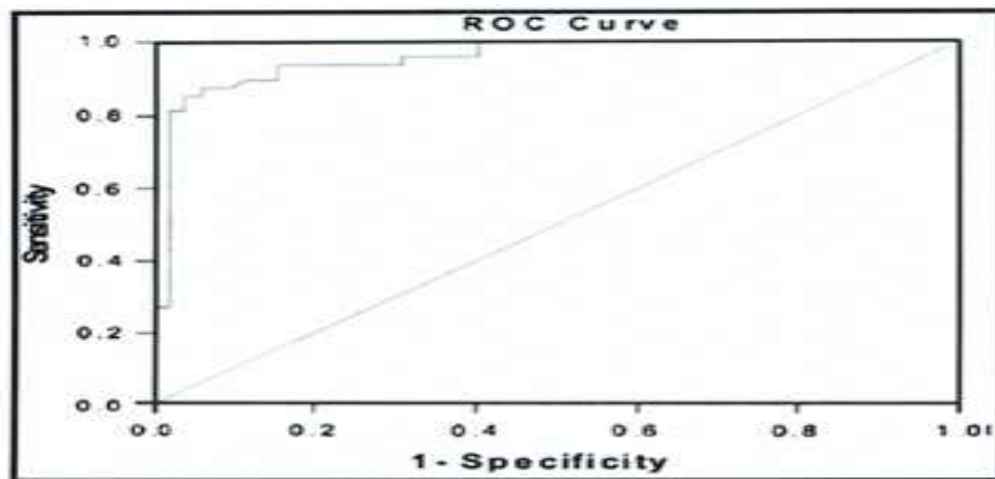


Fig. 3-1 Receiver operator characteristic (ROC) curve chosen lactate cut-off values for diagnosis of suspected PROM as (5mmol/L) with sensitivity of(94%) and specificity of (92%) p-value (<0.05)

Table(2):sensitivity ,specificity ,positive predictive value and negative predictive value of vaginal lactate according to (5mmol\l) cut-off value

vaginal lactate level (5mmol\L)	(%)
Sensitivity	94%
Specificity	92%
positive predictive value	89%
negative predictive value	91%

Table(3) Lactate level distribution among study groups (n = 80)

Lactate level(mmol\l)	Group(1)	Group(2)	Total
>5	38 (95%)	3(7.5%)	41
<5	2(5%)	37(92.5%)	39
Total	40	40	80

Table (3) shows distribution of various lactate levels in the study groups.

Table (4) Mean time of spontaneous onset of labor within 24hr. and 48hr according to lactate level

Lactate level(mmol\L)	Onset within 24 hrs.	Onset within 48 hrs.	Total
>5	35/41 85.3%	39/41 95.1%	41
<5	4/39 10.2%	8/39 20.5%	39
Total	39	47	80

Table (4) shows that high level of lactate > 5mmol/L is associated with higher onset of labor at 24 and 48 hours.

Table (5)mean time of spontaneous onset of labour within 24hrs. and 28 hrs. according to vaginal pooling of amniotic fluid

Amniotic fluid	Onset within 24 hrs.	Onset within 48 hrs.	Total
Group(1)	25 62.5%	34 85%	40
Group (2)	9 22.5%	13 32.5%	40
Total	34	47	80

Pooling of amniotic fluid is associated with higher onset of labour within 24 hrs. and 48 hrs.

Table (6) Vaginal lactate levels versus vaginal amniotic fluid pooling in detection of spontaneous onset of labour within 48 hrs.

Labor within 48 hr. Lactate level	positive	negative	No.
>5mmol/L	39	2	41
<5mmol/L	8	31	39
(group 1)	34	6	40
(group2)	13	27	40

Table (6) shows that 39 of 41 women with vaginal lactate concentration of >5 mmol/L would have labour within 48 hours, while 34 of 40 women with amniotic fluid pooling would have labour within 48 hours.

Table (7) sensitivity ,specificity, positive predictive value and negative predictive value of vaginal lactate versus amniotic fluid pooling for detection of spontaneous onset of labor within 48 hr.

vaginal Lactate level and amniotic fluid pooling	sensitivity	specificity	positive predictive value	negative predictive value
vaginal lactate	79.4%	95%	93.9%	82.9%
amniotic fluid pooling	67.5%	85%	81%	72.3%

Discussion

It is a well-known fact that the time of onset of spontaneous labor after the rupture of membranes is very important in terms of management decisions as well as proper counseling of patients with suspected PROM regarding interventions which may be necessary for them, dexamethasone administration and tertiary center transport of patient with neonatal care unit.⁽²⁶⁾

Traditionally, the diagnosis of membrane rupture has relied on patient's report of fluid leakage, confirmed by the presence of gross pooling of amniotic fluid in vagina with speculum examination and alkaline vaginal pH detected by nitrazine paper test or the presence of characteristic ferning pattern after microscopic examination of dried vaginal secretions.⁽²⁷⁾ A number of new methods have been described to diagnose PROM such as (lactate, prolactin, BHCG, vaginal fluid urea and creatinine) and

AmniSure. These are very helpful in assessing women with suspected PROM.⁽²⁸⁾

In our study, lactate level of (5mmol/L) is the cut-off to diagnose premature rupture of membranes because of high sensitivity (94%), specificity (92%), positive

predictive value (89%) and negative predictive value (91%). These results approximate that of Jaiswar *et al* (2013) who found that cut-off was >5mmol/L.⁽²⁹⁾

Also our findings support those of Wiberg-Itzel *et al* (2005), who found that lactate test had a sensitivity of (86%), specificity (92%), positive predictive value (92%) and negative predictive value (87%) and a lactate concentration > 4.5 mmol/L is the best cut-off value for positive test.⁽²⁵⁾ El-Sokkary *et al* (2015) found that lactate level of 4.3mmol/l or greater was the best cut-off point and provided a sensitivity of (89.06%), a specificity of (85.71%), a positive predictive value of (87.7%) and a negative predictive value of (87.3%)⁽²⁸⁾ and agree with our results.

Regarding distribution of various vaginal lactate levels, (95%) patients in the group(1) or (with vaginal pooling of amniotic fluid) had lactate levels > 5.0 mmol/L, whereas (7.5%) patients in group(2) or (without vaginal pooling of amniotic fluid) had lactate levels > 5.0 mmol/L, this result consistent with that of Jaiswar *et al* (2013) in which (100%) patients in the group (1) had lactate level >5mmol/L whereas (16.4%) of the group(2) had lactate level >5 mmol/L.⁽²⁹⁾

With lactate levels >5.0 mmol/L and onset of spontaneous labor within 24 hrs. and 48hrs., our results agree with that of Jaiswar *et al* (2013) in which (83.87 %) of women had onset of spontaneous labor within 48 hrs. when vaginal lactate level was >5.0 mmol/L. While in women with lactate levels was <5.0 mmol/L, only (19.6 %) had spontaneous onset within 48 hrs. ⁽²⁹⁾ Also Wiberg- Itzel *et al* (2006) found that (53.3%) of patients had spontaneous onset of labor within 24 hrs. and (71.5 %) patients had spontaneous onset of labor within 48 hrs.. ⁽²⁶⁾

Regarding pooling of amniotic fluid, our results agreed with Jaiswar *et al* (2013) results who found that (62.74 %) of patients with pooling of amniotic fluid had spontaneous onset of labor within 24 hrs. and (82.35 %) of patients within 48 hrs., while only (23.88 %) of patients without pooling of amniotic fluid had onset of labor within 24 h and (31.34 %) within 48 hrs. ⁽²⁹⁾ It also agree with Wiberg-Itzel *et al* (2006) in their study found that among 108 patients with pooling of amniotic fluid, (76.89 %) had spontaneous onset of labor within 24 hrs. and (89.81 %) within 48 hrs.. ⁽²⁶⁾

In this study by holding a comparison between vaginal Lactate

level and amniotic fluid pooling for detection of spontaneous onset of labor within 48hr, the results of it agree with Wiberg-Itzel *et al* (2008) who found by that :vaginal Lactate level had a sensitivity of (87%), a specificity of (93%), a positive predictive value of (83,9%) and a negative predictive value of (95%), while amniotic pooling had a sensitivity of (91%), a specificity of (86%), a

positive predictive value of (72%) and a negative predictive value of (96%) . ⁽³⁰⁾

Conclusion: Measurement of vaginal lactate is reliable, simple and rapid test in diagnosis of PROM and can be used as adjunctive test in equivocal cases.

Recommendations: Large comparative and randomized clinical trials between standard methods in diagnosis of PROM such as vaginal fluid pooling, fern test and new methods such as lactate, prolactin, BHCG, vaginal fluid urea and creatinin and AmniSure to find more specific, sensitive, rapid, applicable, bed side and cheap methods in the diagnosis of PROM.

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