

Vaginal Fluid Lactate: A Method for the Diagnosis of Premature Rupture of Membrane

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Abstract

To assess whether lactate determination in vaginal fluids " Lac-test ", can be used as a diagnostic test for premature rupture of membranes (PROM) and to derive the best cutoff value for a positive test. It was a cross-sectional study performed at Al Azhar Bab El Sheria and Ain Shams Maternity Hospitals. 120 women were subdivided into 80 cases with sure or suspected history of PROM and 40 cases with no history of PROM as a control group for determination of lactate concentrations in vaginal fluid, nitrazine paper test and AFI were analysed. : A lactate concentration ≥ 4.3 mmol/L was found to be the best cut-off value for a positive test. The "Lac-test" had a sensitivity of 89.06%, specificity 85.71%, positive and negative predictive values of 87.7% and 87.3%, respectively. Corresponding values for the nitrazine paper test were 75%, 75%, 77.4% and 72.4%. Likelihood ratio for a positive "Lac-test" was 6.2 and for a negative test 0.1. Corresponding values for the nitrazine paper test were 3 and 0.3 respectively. "Lac-test" is a valid test in cases with suspected PROM and may even be a better predictor than the nitrazine test.

Keywords

Lactate Determination - Premature Rupture of Membranes

I. Introduction

Premature rupture of membranes (PROM) is defined as rupture of fetal membranes before the onset of labour at any time during the

gestational period. It occurs in 4.5-7.6% of pregnant women [1]. Premature rupture of membranes is associated with infectious morbidity in mother and fetus, cord accidents, imminent term or preterm labour. Diagnosis of PROM is easy when the rupture is obvious but difficult or indeed impossible when the rupture is slight [2]. Failure to identify patients with membrane rupture can result in failure to implement obstetric measures. Conversely, the false diagnosis of membrane rupture can lead to inappropriate interventions such as hospitalization or induction of labour.

Traditionally, the diagnosis of PROM has relied on a combination of factors, including the patient's history, identification of gross pooling of amniotic fluid in the vagina, ferning pattern, and a positive nitrazine test [3]. However, in equivocal cases of PROM, the traditional methods have been associated with both false-positive and false-negative results [4].

The absence of a noninvasive "gold standard" test for the diagnosis of membrane rupture has led to the search for the alternative biochemical markers. Any biological test used to establish a correct diagnosis must be reliable, simple and rapid [2].

Biochemical substances, which have high amniotic concentration, e.g. prolactin [5], alphafetoprotein (AFP) [6], insulin like growth factor binding protein-1 (IGFBP-1) [7], fetal fibronectin [8], diamino-oxidase⁹, β -HCG [10], had all been previously studied. The nitrazine test is a pH indicator. Vaginal pH is normally between 4.5 and 5.5, but the presence of amniotic fluid in the vagina increases the pH value [11]. Other tests have been used as markers for rupture of the membranes. Arborization or ferning of vaginal fluid

suggests amniotic rather than cervical fluid. If present, amniotic fluid crystallizes to form a fern-like pattern due to the relative concentrations of sodium chloride, proteins, and carbohydrates in that fluid [12].

Over the past decades, a number of ultrasound methods have been used to measure the amount of amniotic fluid. Phelan and colleagues (1987)[13] described the clinical utility of quantification using the amniotic fluid index (AFI). This is calculated by adding the vertical depths of the largest pocket in each of four equal uterine quadrants.

Lactate has also been reported to occur in high concentration in amniotic fluid, (7-9 mmol/l)[14], which is four to six times higher than in maternal or fetal blood [15]. The source of the amniotic fluid lactate has been suggested to be the fetus, mainly through urine and lung fluid excretion [15].

II. Methods

A cross-sectional study was conducted on 120 pregnant women who attended the casualty department at Al Azhar University Bab El Sheria ana Ain Shams Maternity Hospitals with history of PROM without uterine contractions in between August 2011 and Mars 2015. The study was approved by the local research ethics committee in Al-Azhar university faculty of medicine. After giving informed consent, all the patients underwent ultrasonographic examination for determination of gestational age and calculation of amniotic fluid index. The amniotic fluid index (AFI) was assessed in four quadrants. According to the method of Phelan et al.,(1987) an AFI of < 8cm was considered as having oligohydroamnios. Pooling of amniotic fluid in the posterior vaginal fornix

during speculum examination was used as the gold standard in order to be able to calculate the sensitivity, specificity, positive predictive value and negative predictive value of each test. **After that the patients were divided into two groups:**

Group I :(n= 80) patients with sure or suspected history of PROM (study group) which were subdivided into:

Group Ia:(n=40) patients with sure history of PROM confirmed by gush of fluid, ultrasonographic amniotic fluid index (AFI).

Group Ib:(n=40) patients with suspected history of PROM as they gave history of leakage of fluid , with or without decrease in AFI.

Group II: (n=40) patients with no history of PROM, normal AFI (control group).

• **Inclusion criteria:**

- 1- Suspected or sure history of PROM.
- 2- Absence of uterine contractions, fetal distress or cord prolapse.
- 3- Absence of vaginal discharge or previous recent vaginal treatment.

• **Exclusion criteria:**

- 1- Presence of uterine contractions, fetal distress or cord prolapse.
- 2- Fever.
- 3- Infected vaginal discharge or previous recent vaginal treatment.

At the time of speculum examination, two samples of vaginal fluid were aspirated. One sample was used for lactate determination and the other sample for the nitrazine paper test which is a pH indicator. The vaginal pH is normally between 4.5 and 5.5, so the presence of amniotic fluid in the vagina will increase the pH value. The test was interpreted as positive when the test paper turns blue.

For lactate determination, the commercially available Lactate Pro. (an electrochemical test strip method) was used an (accutrend® lactate) type 3012522 an electrochemical equipment of Roche company and we used (BM–Lactate) as test strips for the quantitative determination of lactate in amniotic fluid. The test needed only 5 µl of fluid for analysis. It was carried out at the bedside and results were available after 60 seconds.

Reaction Principle:

- Each test strip has a test area containing detection reagents. When amniotic fluid is applied, a chemical reaction takes place and the test area changes colour. The (Accutrend® Lactate) records this change in colour and converts the measured signal to the displayed result using the data previously entered by means of the code strip.
- The applied amniotic fluid seeps through the yellow protective mesh into a glass fibre fleece. Lactate is determined by reflectance photometry at a wave length of 657 nm via a colorimetric lactate-oxidase mediator reaction. L-lactate + mediator form I — LOD → pyruvate + mediator reduced. Mediator reduced +2,18-phosphomolybdate → molybdane blue + mediator form II
- Components per test: lactate oxidase (rec.Aerococcus viridans) 1,9 U;N,N-bis-(2-hydroxyethyl)-4-hydroximinocyclohexa-2,5-dienylidene)ammonium-chloride 7.2 µg; phosphomolybdate 11.4 µg.
- Performance characteristics: The data for BM-Lactate were determined in series of tests during evaluation. The

majority of the data for the test were within the given ranges. Repeatability (within-series imprecision): CV (coefficient of variation) 5.5 % in the normal range, 5 % in the higher range. Reproducibility (day-to-day imprecision). CV 4.8 % in the low range, 3.3 % in the pathological range; sample material: control solutions. Accuracy (methods comparisons, mmol/l: regression equations, n samples, correlation coefficients r) $y = 0.957 x - 0.042$ and $1.039 x + 0.325$, respectively, (n = 77-147, r = 0.970), reference method x: Test Combination Lactate, Roche Diagnostics. Detection limit (lowest value detected) 0.8 mmol/l and 0.7 mmol/l. respectively.

III. Results

The clinic-epidemiological characteristics of patients in the three study groups were studied in table 1. There was no statistically significant difference among the three groups as regards the gestational age (p-value, 0.870), parity (p-value, 0.933), number of previous abortions (p-value, 0.626) or the mode of delivery (p-value, 0.074)

Table 2 and Figure 1 show the results of receiver-operating characteristic (ROC) curve analysis for the diagnosis of ruptured membranes using vaginal lactate. Vaginal lactate had very good diagnostic value as evidenced by an area under the ROC curve (AUC) of 0.856 (95% CI, 0.780 - 0.913; p-value, <0.0001). The best cut-off criterion was a vaginal lactate level of >4.3 mmol/l (Youden index, 0.748; 95% CI 0.609 - 0.851). This had a

sensitivity of 89.1% (95% CI, 78.8% - 95.5%), a specificity of 85.7% (95% CI, 73.8% - 93.6%), a +LR of 6.2 (95% CI, 3.3 - 11.9), a -LR of 0.1 (95% CI, 0.1 - 0.3), a +PV of 87.7% (95% CI, 77.2% - 94.5%), and a -PV of 87.3% (95% CI, 75.4% - 94.8%).

The prevalence of a vaginal lactate level of >4.3 mmol/l in the three groups was studied. 34 women (85%) in the visible ROM group had a vaginal lactate level of >4.3 mmol/l compared with 23 (57.5%) and 8 (20%) patients in the suspected ROM group and control group, respectively with statistically significant difference (p-value <0.001) as shown in figure 2.

The results of receiver-operating characteristic (ROC) curve analysis for the diagnosis of ruptured membranes using the AFI were shown in table 3 and figure 3. The AFI had excellent diagnostic value as evidenced by an area under the ROC curve (AUC) of 0.951 (95% CI, 0.896 - 0.982; p-value, <0.0001). The best cut-off criterion was an AFI of ≤ 8 (Youden index, 0.746; 95% CI 0.609 - 0.819). This had a sensitivity of 78.1% (95% CI, 66.0% - 87.5%), a specificity of 96.4% (95% CI, 87.7% - 99.6%), a +LR of 21.9 (95% CI, 5.6 - 85.8), a -LR of 0.2 (95% CI, 0.1 - 0.4), a +PV of 96.2% (95% CI, 86.7% - 99.5%), and a -PV of 79.4% (95% CI, 67.9% - 88.3%). The prevalence of an AFI of ≤ 8 in the three study groups was described in figure 4. Twenty-eight (70%) patients in the Visible ROM group had an AFI of ≤ 8 compared with 23 (57.5%) patients and 1 (2.5%) patient in the Suspected ROM group and Control group, respectively. These differences were statistically significant (p-value <0.001).

The analysis of the ROC curve derived from the predicted probability for ROM as estimated

from the simple logistic regression model using a positive nitrazine test as a marker was described in table 4 and figure 5. A positive nitrazine test had a good diagnostic value as evidenced by an area under the ROC curve (AUC) of 0.75 (95% CI, 0.663 - 0.825; p-value, <0.0001). The best cut-off criterion was a predicted probability of >0.276 (Youden index, 0.5; 95% CI, 0.335 - 0.648). This had a sensitivity of 75% (95% CI, 62.6% - 85.0%), a specificity of 75% (95% CI, 61.6% - 85.6%), a +LR of 3 (95% CI, 1.9 - 4.8), a -LR of 0.3 (95% CI, 0.2 - 0.5), a +PV of 77.4% (95% CI, 65.0% - 87.1%), and a -PV of 72.4% (95% CI, 59.1% - 83.3%). The prevalence of a positive nitrazine test in the study groups was presented in figure 6. 34 women (82.5%) in the Visible ROM group had a positive nitrazine test compared with 17 (42.5%) and 12 (30%) women in the suspected ROM group and control group, respectively. These differences were statistically significant (p-value <0.001). The analysis of the ROC curve derived from the predicted probability for ROM as estimated from the multivariable binary logistic regression model using a vaginal lactate level of >4.3 mmol/l, a positive nitrazine test, and an AFI of ≤8 combined was presented in table 5 and figure 7. The model had an excellent diagnostic value as evidenced by an area under the ROC curve (AUC) of 0.979 (95% CI, 0.934 - 0.996; p-value, <0.0001). The best cut-off criterion was a predicted probability of >0.366 (Youden index, 0.871; 95% CI, 0.775 - 0.951). This had a sensitivity of 90.6% (95% CI, 80.7% - 96.5%), a specificity of 96.4% (95% CI, 87.7% - 99.6%), a +LR of 25.4 (95% CI, 6.5 - 99.2), a -LR of 0.1 (95% CI, 0.1 - 0.2), a +PV of 96.7% (95% CI, 88.4% - 99.6%), and a -PV of 90% (95% CI, 79.5% - 96.2%). Figure 8

shows a comparison of the areas under the ROC curves (AUCs) for vaginal lactate, AFI, nitrazine test, or all three markers combined. There was no statistically significant difference between the AUC associated with the combination of the three markers and the AUC associated with the AFI (p-value, 0.055). However, the AUC associated with the combination of the three markers was significantly larger than that associated with vaginal lactate (p-value, <0.001) and that associated with a positive nitrazine test (p-value, <0.0001). The AUCs associated with vaginal lactate and the AFI were significantly larger than that associated with a positive nitrazine test (p-value, 0.024 and p-value <0.0001, respectively). The difference between the AUC associated with vaginal lactate and that associated with the AFI was not statistically significant (p-value, 0.067). Table 6 and Figure 9 show the results of the Kaplan-Meier analysis for the time to onset of labor. The median time to onset of labor was 25 h in women with vaginal lactate of ≤4.3 mmol/l compared with 10 h in those with vaginal lactate of >4.3 mmol/l with a hazard ratio of 3.9 (95% CI, 2.1 to 7.0; p-value, <0.0001).

IV. Discussion

The diagnosis of premature rupture of membranes in premature pregnancy allows estimating the dangers which threaten both the fetus and the pregnant woman and helps to put into practice the most accurate therapeutic procedures [17]. 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 [2].

Cytological staining techniques for identification of fetal lanugo, fat globules and squamous cells are diagnostic tests that are no longer used because they take time, technically difficult and they are not found in all units and their false-negative rate is high [18]. AFI volume measurement might be used in the diagnosis of PROM as well as having a prognostic value. AFI is decreased if a large volume of amnion has leaked [19].

This study is a cross sectional study which was done to assess whether lactate determination in vaginal fluids 'lac-test' can be used as a diagnostic test for premature rupture of membranes (PROM) and to drive the best cut-off value for a positive test.

The study was carried out at Al Azhar University Bab El Sheria and Ain Shams Maternity Hospitals. 120 consenting pregnant women after 37 wks, attending the hospital were recruited in the current study, of whom 40 (30%) met the criteria for sure rupture of membranes as a PROM group (group Ia), 40 (30%) with suspected PROM (group Ib) and 40 (30%) without rupture of membranes as a control group (group II). Pooling of amniotic fluid in the posterior vaginal fornix during speculum examination was used as the gold standard in order to be able to calculate the sensitivity, specificity, positive predictive value and negative predictive value of each test. There was no statistically significant differences in maternal age, gestational age at membranes rupture, and the numbers of deliveries and abortions.

In this study we compared three markers: lactic acid, nitrazine test, and amniotic fluid index (AFI) for the diagnosis of PROM. In all patients, amniotic fluid index (AFI) was assessed according to the method of Phelan et al., (1987) [13]. Comparison between group Ia, Ib and group II showed that there was a statistically significant difference between the three groups as regards the mean value of AFI, group Ia and group Ib showed a statistically significant lower AFI more than group II. Amniotic fluid index (AFI) was statistically lower among PROM group (group Ia) compared to control group (group II).

Erdemoglu and Mungan (2004) [11] demonstrated that AFI values of less than 5 cm and 5-8 cm is usually accepted as definite oligohydramnios and borderline oligohydramnios, respectively. They found that AFI less than 8 cm had 94% sensitivity, 91% specificity.

Martinez et al., (2006) [20] observed that a reduced amount of amniotic fluid might represent other pregnancy complications, such as placental insufficiency. They found that AFI less than 5 cm had 19% diagnostic sensitivity, 100% diagnostic specificity, 100% PPV and 61% NPV. An AFI < 5cm at admission has been found to be a useful prognostic variable in the management of third trimester pregnancies affected by PPRM.

Ultrasonographic AFI determination is helpful but not reliable, because oligohydramnios for any reason cannot be distinguished easily from decreased amniotic fluid volume as a result of PROM. Also, in cases of minor membrane rupture and amniotic fluid drainage, amniotic fluid volume may be normal. Therefore, false-positive and false-negative rates are high [21].

The mean lactate concentration in the vaginal fluids of women with sure PROM, (group Ia) and those with suspected PROM (group Ib) were significantly higher than that of the control group (group II), while there was no statistically significant difference between the means of lactate concentration in the vaginal fluids of group Ia and group Ib in agreement with previous studies [22].

Although, group Ia showed significantly higher percentage of +ve results of nitrazine test than group Ib and both groups showed a significantly higher percentage of +ve test than the control group (group II). These findings support the data reported in the previous studies [23]. But the reliability of nitrazine paper test is poor after 48 hrs. Moreover, cervicitis, vaginitis, contamination of vagina with alkaline urine, semen, blood and antiseptics is associated with false (+ve) nitrazine paper test [24]. The use of an acidity indicator, such as nitrazine sticks (Amnicator, Corsham), is not reliable, as this indicates only that the vagina is no longer acidic, an effect that can be produced by urine or bath water.

In the present study, lactate level of 4.3mmol/L or greater provided a sensitivity of 89.06%, a specificity of 85.71% and a positive predictive value of 87.7%, negative predictive value of 87.3% was the best cut off point to diagnose premature rupture of membranes. The main difference between the "Lac test" and the nitrazine paper test was the specificity: when there was visible amniotic fluid, the specificity of the "Lac test" was 85.71%, while the specificity for the nitrazine test was 75%. The sensitivity of "Lac test" (i.e. the prevalence of a positive "Lac test") with actual PROM was 89.06%, while the sensitivity for the nitrazine test was 75%.

Our findings support those of Wiberg-Itzel et al., (2005) [25] who found that lactate test had a sensitivity of 86%, specificity of 92%, positive predictive value of 92% and negative predictive value of 87% and a lactate concentration > 4.5 mmol/L as the best cut-off value for +ve test.

For lactate determination, the commercially available Lactate Pro, was used in this study. The test needs only 5 µl of amniotic fluid for the analysis. It is carried out at the bedside and the result will be available after 60 seconds. Furthermore; our study supports those of Wiberg-Itzel et al., (2006) [26], that lactate in amniotic fluid can be used in the prediction of spontaneous onset of labour for women with suspected PROM. High lactate concentration > 4.3 mmol/L in the vaginal fluids can be used to predict whether a woman with suspected PROM will commence spontaneous onset of labour within 4h to 48h

V. Conclusion

The detection of lactic acid in the vaginal fluid is a rapid, reliable and noninvasive method for diagnosis of premature rupture of membranes. Unlike other tests, the test is not affected by semen, vaginal discharge or the length of time from membranes rupture to the application of the test. The simplicity, the accuracy of the lactate pro, being a quick bedside method, makes it suitable in clinical practice for the diagnosis of PROM.

. Competing interest

No competing interests to declare about this work

Author contributions

All authors were included in conception, design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis and supervision

VI. References

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Table 1. Patients' characteristics of the three studied groups

Variable		Control (n=40)	Visible ROM (n=40)	Suspected ROM (n=40)	P value
Gestational age	37-40 wk	39 (97.5%)	37 (92.5%)	38 (95.0%)	0.870
	>40 wk	1 (2.5%)	3 (7.5%)	2 (5.0%)	
Parity	P0	16 (40.0%)	13 (32.5%)	14 (35.0%)	0.933
	P1	9 (22.5%)	8 (20.0%)	13 (32.5%)	
	P2	8 (20.0%)	10 (25.0%)	7 (17.5%)	
	P3	5 (12.5%)	4 (10.0%)	5 (12.5%)	
	P4	2 (5.0%)	2 (5.0%)	0 (0.0%)	
	P5	0 (0.0%)	3 (7.5%)	1 (2.5%)	
Previous abortion	Nil	26 (65.0%)	29 (72.5%)	28 (70.0%)	0.626
	One	9 (22.5%)	7 (17.5%)	9 (22.5%)	
	Two	2 (5.0%)	2 (5.0%)	1 (2.5%)	
	Three	2 (5.0%)	2 (5.0%)	0 (0.0%)	
	Four or more	1 (2.5%)	0 (0.0%)	2 (5.0%)	
Mode of delivery	Cesarean	-	7 (17.5%)	9 (37.5%)	0.074
	Vaginal delivery	-	33 (82.5%)	15 (62.5%)	

Data are presented as number (%).

Table 1 shows characteristics of patients in the three study groups. There was no statistically significant difference among the three groups as regards the gestational age (p-value, 0.870), parity (p-value, 0.933), number of previous abortions (p-value, 0.626) or the mode of delivery (p-value, 0.074)

Table 2

	Estimate	95% CI	P value
AUC	0.856	0.780 - 0.913	<0.0001
Youden index J	0.748	0.609 - 0.851	
Associated criterion	>4.3	3.4 - 4.3	
Sensitivity, %	89.1	78.8 - 95.5	
Specificity, %	85.7	73.8 - 93.6	
+LR	6.2	3.3 - 11.9	
-LR	0.1	0.1 - 0.3	
+PV, %	87.7	77.2 - 94.5	
-PV, %	87.3	75.4 - 94.8	

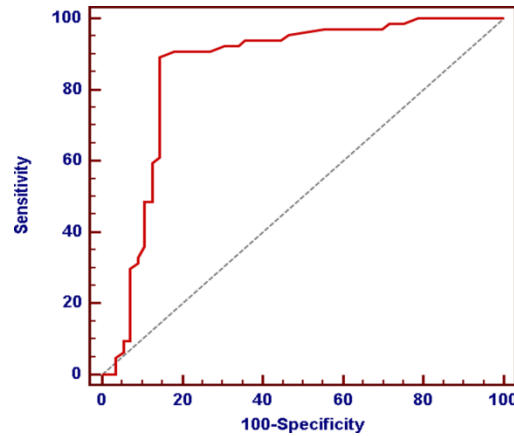


Figure (1)

Table 2 and **Figure 1** show the results of receiver-operating characteristic (ROC) curve analysis for the diagnosis of ruptured membranes using vaginal lactate. Vaginal lactate had very good diagnostic value as evidenced by an area under the ROC curve (AUC) of 0.856 (95% CI, 0.780 - 0.913; p-value, <0.0001). The best cut-off criterion was a vaginal lactate level of >4.3 mmol/l (Youden index, 0.748; 95% CI 0.609 - 0.851). This had a sensitivity of 89.1% (95% CI, 78.8% - 95.5%), a specificity of 85.7% (95% CI, 73.8% - 93.6%), a +LR of 6.2 (95% CI, 3.3 - 11.9), a -LR of 0.1 (95% CI, 0.1 - 0.3), a +PV of 87.7% (95% CI, 77.2% - 94.5%), and a -PV of 87.3% (95% CI, 75.4% - 94.8%).

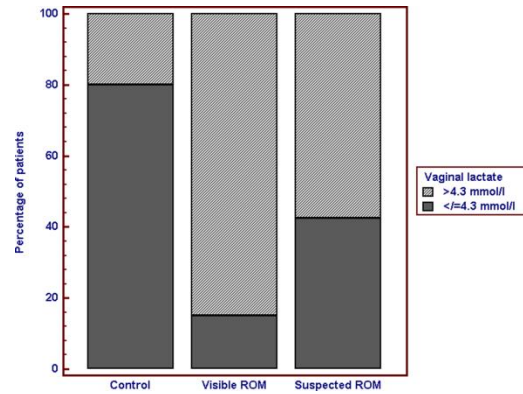


Figure (2)

Figure 2 shows the prevalence of a vaginal lactate level of >4.3 mmol/l in the three groups. 34 women (85%) in the visible ROM group had a vaginal lactate level of >4.3 mmol/l compared with 23 (57.5%) and 8 (20%) patients in the suspected ROM group and control group, respectively with statistically significant difference (p-value <0.001).

	Estimate	95% CI	P value
AUC	0.951	0.896 - 0.982	<0.0001
Youden index J	0.746	0.609 - 0.819	
Associated criterion (probability)	≤8	6.461 - 9.0	
Sensitivity, %	78.1	66.0 - 87.5	
Specificity, %	96.4	87.7 - 99.6	
+LR	21.9	5.6 - 85.8	
-LR	0.2	0.1 - 0.4	
+PV, %	96.2	86.7 - 99.5	
-PV, %	79.4	67.9 - 88.3	

Table 3

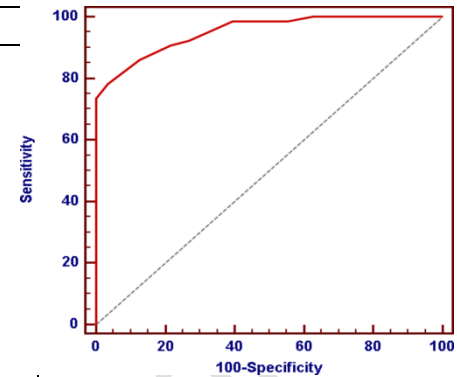


Figure (3)

Table 3 and **Figure 3** show the results of receiver-operating characteristic (ROC) curve analysis for the diagnosis of ruptured membranes using the AFI. The AFI had excellent diagnostic value as evidenced by an area under the ROC curve (AUC) of 0.951 (95% CI, 0.896 - 0.982; p-value, <0.0001). The best cut-off criterion was an AFI of ≤8 (Youden index, 0.746; 95% CI 0.609 - 0.819). This had a sensitivity of 78.1% (95% CI, 66.0% - 87.5%), a specificity of 96.4% (95% CI, 87.7% - 99.6%), a +LR of 21.9 (95% CI, 5.6 - 85.8), a -LR of 0.2 (95% CI, 0.1 - 0.4), a +PV of 96.2% (95% CI, 86.7% - 99.5%), and a -PV of 79.4% (95% CI, 67.9% - 88.3%).

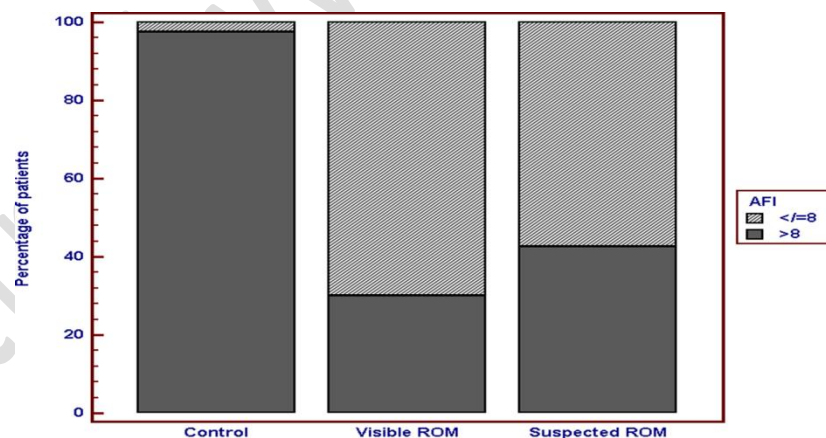


Figure (4)

Figure 4 shows the prevalence of an AFI of ≤ 8 in the three study groups. Twenty-eight (70%) patients in the Visible ROM group had an AFI of ≤ 8 compared with 23 (57.5%) patients and 1 (2.5%) patient in the Suspected ROM group and Control group, respectively. These differences were statistically significant (p-value < 0.001).

	Estimate	95% CI	P value
AUC	0.75	0.663 - 0.825	< 0.0001
Youden index J	0.5	0.335 - 0.648	
Associated criterion	> 0.276	0.276 - 0.276	
Sensitivity, %	75	62.6 - 85.0	
Specificity, %	75	61.6 - 85.6	
+LR	3	1.9 - 4.8	
-LR	0.3	0.2 - 0.5	
+PV, %	77.4	65.0 - 87.1	
-PV, %	72.4	59.1 - 83.3	

Table 4

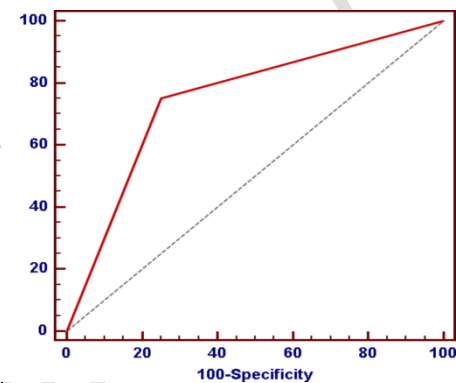


Figure (5)

Table 4 and **Figure 5** show the analysis of the ROC curve derived from the predicted probability for ROM as estimated from the simple logistic regression model using a positive nitrazine test as a marker. A positive nitrazine test had a good diagnostic value as evidenced by an area under the ROC curve (AUC) of 0.75 (95% CI, 0.663 - 0.825; p-value, < 0.0001). The best cut-off criterion was a predicted probability of > 0.276 (Youden index, 0.5; 95% CI, 0.335 - 0.648). This had a sensitivity of 75% (95% CI, 62.6% - 85.0%), a specificity of 75% (95% CI, 61.6% - 85.6%), a +LR of 3 (95% CI, 1.9 - 4.8), a -LR of 0.3 (95% CI, 0.2 - 0.5), a +PV of 77.4% (95% CI, 65.0% - 87.1%), and a -PV of 72.4% (95% CI, 59.1% - 83.3%).

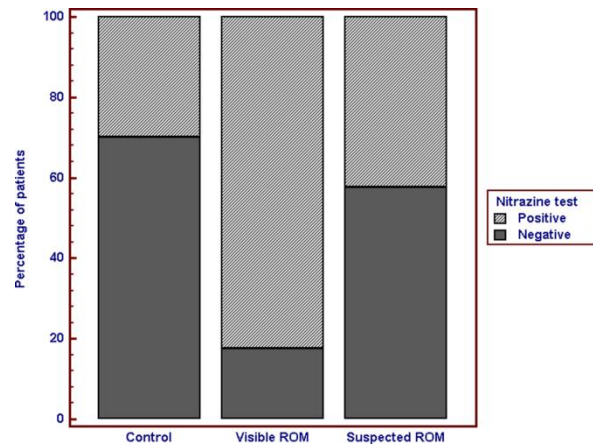


Figure (6)

Figure 6 shows the prevalence of a positive nitrazine test in the study groups. 34 women (82.5%) in the Visible ROM group had a positive nitrazine test compared with 17 (42.5%) and 12 (30%) women in the suspected ROM group and control group, respectively. These differences were statistically significant (p-value <0.001).

	Estimate	95% CI	P value
AUC	0.979	0.934 - 0.996	<0.0001
Youden index J	0.871	0.775 - 0.951	
Probability	>0.366	0.152 - 0.366	
Sensitivity, %	90.6	80.7 - 96.5	
Specificity, %	96.4	87.7 - 99.6	
+LR	25.4	6.5 - 99.2	
-LR	0.1	0.1 - 0.2	
+PV, %	96.7	88.4 - 99.6	
-PV, %	90	79.5 - 96.2	

Table 5

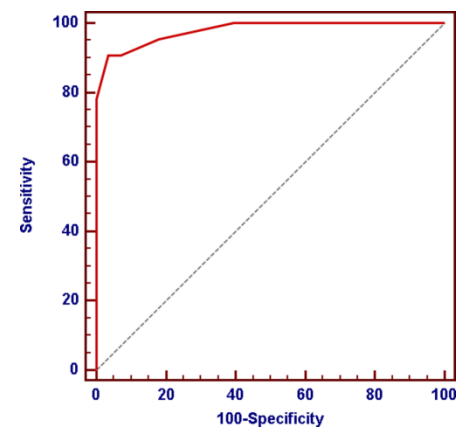


Figure (7)

Table 5 and **Figure 7** show the analysis of the ROC curve derived from the predicted probability for ROM as estimated from the multivariable binary logistic regression model using a vaginal lactate level of >4.3 mmol/l, a positive nitrazine test, and an AFI of ≤8 combined. The model had

an excellent diagnostic value as evidenced by an area under the ROC curve (AUC) of 0.979 (95% CI, 0.934 - 0.996; p-value, <0.0001). The best cut-off criterion was a predicted probability of >0.366 (Youden index, 0.871; 95% CI, 0.775 - 0.951). This had a sensitivity of 90.6% (95% CI, 80.7% - 96.5%), a specificity of 96.4% (95% CI, 87.7% - 99.6%), a +LR of 25.4 (95% CI, 6.5 - 99.2), a -LR of 0.1 (95% CI, 0.1 - 0.2), a +PV of 96.7% (95% CI, 88.4% - 99.6%), and a -PV of 90% (95% CI, 79.5% - 96.2%).

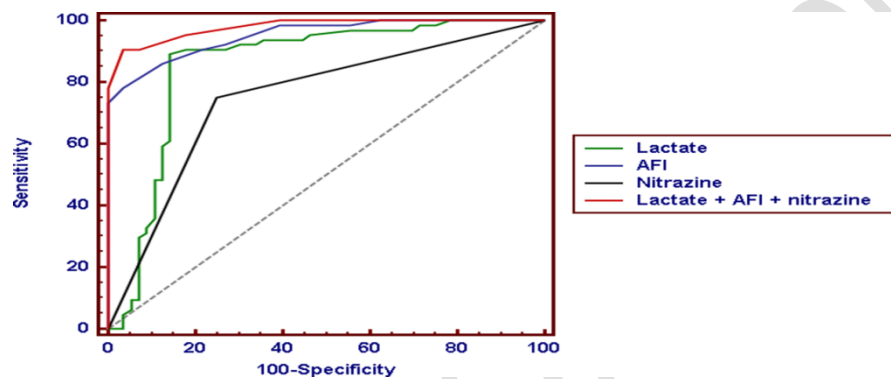


Figure (8)

Figure 8 shows a comparison of the areas under the ROC curves (AUCs) for vaginal lactate, AFI, nitrazine test, or all three markers combined. There was no statistically significant difference between the AUC associated with the combination of the three markers and the AUC associated with the AFI (p-value, 0.055). However, the AUC associated with the combination of the three markers was significantly larger than that associated with vaginal lactate (p-value, <0.001) and that associated with a positive nitrazine test (p-value, <0.0001). The AUCs associated with vaginal lactate and the AFI were significantly larger than that associated with a positive nitrazine test (p-value, 0.024 and p-value <0.0001, respectively). The difference between the AUC associated with vaginal lactate and that associated with the AFI was not statistically significant (p-value, 0.067).

	Vaginal lactate ≤4.3 mmol/l (n=55)	Vaginal lactate >4.3 mmol/l (n=65)
Logrank test		
Observed number with onset of labor	6	42
Expected number with onset of labor	17.1	30.9
Chi-square	19.723	
DF	1	
P value	< 0.0001	
Median time to onset of labor (hr)	25.5	10
Hazard ratio	3.9 (95% CI, 2.1 to 7.0)	

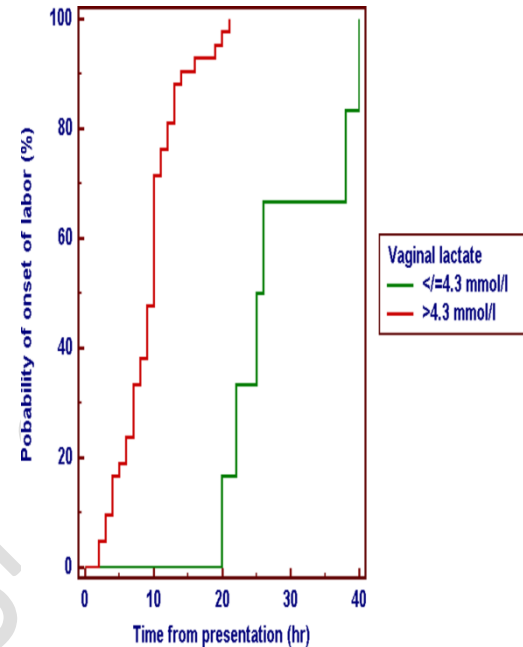


Table 6

Figure(9)

Table 6 and **Figure 9** show the results of the Kaplan-Meier analysis for the time to onset of labor. The median time to onset of labor was 25 h in women with vaginal lactate of ≤4.3 mmol/l compared with 10 h in those with vaginal lactate of >4.3 mmol/l with a hazard ratio of 3.9 (95% CI, 2.1 to 7.0; p-value, <0.0001).