RESEARCH ARTICLE

Beta hCG, urea and creatinine levels in vaginal fluids: a reliable marker for prelabour rupture of membranes

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ABSTRACT

Objective: To study the levels of β-hCG, urea and creatinine in vaginal fluid for diagnosis of prelabour rupture of membranes (PROM). **Methodology:** This was observational cross sectional comparative study divided into two groups. Group A (cases)- Pregnant women with gestational age 28-40 weeks presenting with history of leaking per vaginum without onset of labor pains, and who are clinically diagnosed as prelabor rupture of membranes. Group B (control) - Pregnant women with gestational age 28-40 weeks without PROM. T-test and chi-square test was used to calculate and to measure a quantitative and qualitative variables. Receiver operating characteristic curve was be used to determine a cut-off value. Cut-off point was set at highest optimal sensitivity and specificity. **Results**: Demographic data of both groups were comparable. Vaginal fluid value of β-hCG, urea and creatinine was significantly higher in study group. The sensitivity, specificity and cut-off value of vaginal fluid β-hCG 95%, 83.3% and >9 mg/dl respectively. The sensitivity, specificity and cut-off value of vaginal fluid urea/creatinine were 63.33/86.67%, 98.3/48.3% and >3.4/>0.08 IU/L respectively. The area under curves was 0.967 for β-hCG, 0.849 for urea, 0.742 for creatinine and 0.838 for AFI. **Conclusions**: Detection of vaginal fluid β-hCG, urea and creatinine to diagnose PROM is a simple, reliable and rapid test. So, introduction of these methods into routine use even in low resources community settings is feasible, practical and cost-effective.

Keywords: Prelabour rupture of membrane (PROM), β-hCG, urea, creatinine, vaginal.

Rupture of the fetal membranes prior to the onset of labor, regardless of gestational age is defined as PROM[1]. Accurate history of watery discharge per vaginum with collection of fluid in posterior fornix on speculum examination forms mainstay of diagnosis of PROM. Over or under diagnosis of PROM may lead to inappropriate management and unnecessary obstetric interventions which can lead to serious maternal and neonatal complications [2].

Diagnosis of PROM is often made firstly by observation of clear amniotic fluid flow or accumulation of fluid in posterior fornix on speculum examination, secondly by Nitrazine test: pH indicator paper turning from yellow to blue colour which indicates amniotic fluid which has a basic pH and thirdly by Fern test : microscopic appearance of palm leaf-pattern of dried amniotic fluid. However, these traditional methods have many flaws [3].

In early pregnancy the fetal membranes are resistant to rupture. Near term, membranes becomes weaker and also the collagen content in membrane decreases which increases the rupture risk[2]. Beside these, the fetal movements, increase in uterine tension and uterine contractions facilitates the rupture of membranes at term. PROM occurs in 10% of all term pregnancies and about 2-4% of preterm pregnancies leading to infection and preterm deliveries.

History from patients is reliable in 10% to 50% of cases and speculum examination of fluid leakage from the cervix is associated with 12% to 30% false negative results. . Unfortunately, Nitrazine test has a high false positive rate as urine, meconium, semen, blood, cervicitis, vaginitis or any antiseptic agents present in cervicovaginal discharge can

interfere with result as all have alkaline nature. Fern test is also associated with both false positive and false negative results [4]. Even, amniotic fluid estimation by USG examination may mislead us because it cannot differentiate PROM from other causes of decreased liquor [5].

Invasive test like intra amniotic dye injection carries increased maternal and fetal risk [6]. The Amnisure ROM test is another new test that's easy, fast, and minimally invasive with high sensitivity and specificity [6-8]. However, Amnisure is not available in many centres in India and it is an expensive procedure. Recently, detection of urea, creatinine and β-hCG in cervicovaginal discharge has been advised. Fetal urine is the main component of amniotic fluid in the 2nd half of pregnancies, as fetus starts excreting urine into the amniotic fluid at 8th to 11th week of gestation which is the basis for use of urea and creatinine in diagnosing PROM [9, 10]. β- human chorionic gonadotrophin (β-hCG), is secreted solely by syncytiotrophoblasts and can be found in amniotic fluid in addition to mother's blood or urine and has been studied for the evaluation [11].

Materials and methods

This is an observational cross sectional comparative study done at department of Obstetrics and Gynaecology ESI-PGIMSR, Basaidarapur, New Delhi from November 18 to February 2020. The sample was divided into 2 groups; Group A : Pregnant women with gestational age 28-40 weeks presenting with history of leaking per vaginum without onset of labor pains, and who are clinically diagnosed as prelabor rupture of membranes; Group B: Pregnant women with gestational age 28-40 weeks without PROM.

The sample size was calculated based on accuracy level of diagnosis of PROM by the measurement of vaginal fluid β-hCG, urea and creatinine, which was found to be 85% in the previous studies. Hence, from the available information the sample size is calculated to 120 with 90% of power at 95% confidence level. Pregnant women with gestational age - 28 to 40 weeks (with reliable LMP or with USG confirmed dates), single intrauterine pregnancy and consent for participation in study were under inclusion criteria. Multiple pregnancy, placenta praevia, blood in vaginal discharge, insulin dependent DM/ Nephropathy, intrauterine death, if regular uterine contractions associated or any congenital malformations were excluded.

Detailed history was taken alongwith general and abdominal examination as well as sterile speculum examination was done to confirm amniotic fluid flowing

from the cervix from all the women who participated in study. A cotton tip applicator was inserted in deep vagina which was transferred on pH paper. Then, pH paper turned to blue color from yellow; it was considered as positive for amniotic fluid leak from the cervix. Group A included 60 pregnant women, who had complained of vaginal fluid leakage, and in whom PROM was diagnosed via positive fluid leak upon sterile speculum examination and positive pH paper test. And rest, 60 with negative leakage, with matched gestational age, negative fluid leakage upon sterile examination, no change in pH paper test, were in control (Group B). All patients were evaluated for gestational age, amniotic fluid index, and fetal viability by transabdominal sonography. Vaginal aspirate was then collected as following: 5 ml of sterile saline solution was injected into the posterior vaginal fornix and 3 ml of it, was withdrawn with the same syringe.

Sampling procedure was performed within 6 hours after membrane rupture and before vaginal examination or administration of any drugs. Samples were sent to the laboratory for measurement of β-hCG, urea and creatinine. β-hCG was estimated by electrochemi-lumiscene test. Urea level was measured by enzymatic urease method and creatinine level was detected by Jaffe's method respectively.

Demographic and obstetric characteristics, results of speculum examination, pH test, amniotic fluid β-hCG, urea and creatinine levels, as well as ultrasonographic finding of AFI was documented in a data form .

Statistical analysis: T-test and chi-square test was used to calculate and to measure a quantitative and qualitative variables. Receiver operating characteristic curve was used to determine a cut-off value. Cut-off point was set at highest optimal sensitivity and specificity. Data was analysed using SPSS version 21 (Chicago). P value < 0.05 was considered significant.

Results

A total 120 pregnant participants were included in the study, divided equally into cases and control. Demographic data for study groups was calculated and we found that mean age of patients in non-PROM and PROM 26.92±3.39 and 26.32±3.00 years respectively. In non-PROM group as well as PROM group, majority of cases 66% and 50% respectively were from 36-39 weeks of gestational age. In PROM group, the majority of the women were primigravida, followed by gravida 2 and gravida 3, though it does not hold any significance in our study.

In non-PROM group mean β-hCG level was 6.27 ± 7.21 IU/l and in the PROM group it was 282.13 ± 541.07 IU/l. The difference was found to be statistically significant (p=0.001), showing a significantly higher level of β-hCG in the PROM group in comparison to the non-PROM group (table1).

Table 1: Comparison of beta-hCG between the two groups

Groups	Beta-hCG level (Mean ± SD)	't' value	P value
No-PROM group	6.27 ± 7.21	-3.949, df=118	0.001*
PROM group	282.13 ± 541.07		
Unpaired 't' test applied. P value < 0.05 was taken as statistically significant.			

Similarly, in the non-PROM group, the mean vaginal fluid urea level was 1.11 ± 0.97 mg/dL and in the PROM group it was 10.80 ± 12.76 mg/dL. The difference was found to be statistically significant (p=0.001), showing a significantly higher level of vaginal fluid urea level in the PROM group in comparison to the non-PROM group (table 2).

Table 2: Comparison of serum urea level between the two groups

Groups	Serum urea level (Mean ± SD)	't' value	P value
No-PROM group	1.11 ± 0.97	-5.858, df=118	0.001*
PROM group	10.80 ± 12.76		
Unpaired 't' test applied. P value < 0.05 was taken as statistically significant.			

In the non-PROM group, the mean vaginal fluid creatinine level was 0.25 ± 0.29 mg/dL and in the PROM group it was 0.70 ± 0.72 mg/dL. The difference was found to be statistically significant (p=0.001), showing a significantly higher level of vaginal fluid creatinine level in the PROM group in comparison to the non-PROM group (table 3).

Table 3: Comparison of serum creatinine level between the two groups

Groups	Serum creatinine level (Mean ± SD)	't' value	P value
No-PROM group	0.25 ± 0.29	-4.532,df=118	0.001*
PROM group	0.70 ± 0.72		
Unpaired 't' test applied. P value < 0.05 was taken as statistically significant.			

In the non-PROM group, the mean amniotic fluid index was 10.14 ± 3.02 cm and in the PROM group it was 6.69 ± 2.11cm. The difference was found to be statistically significant (p=0.001), showing a significantly lower amniotic fluid index in the PROM group in comparison to the non-PROM group (table 4).

Table 4: Comparison of amniotic fluid index between the two groups

Groups	Amniotic Fluid Index (Mean ± SD)	't' value	P value
No PROM group	10.14 ± 3.02	7.234, df=118	0.001*
PROM group	6.69 ± 2.11		
Unpaired 't' test applied. P value < 0.05 was taken as statistically significant.			

The ROC curves showed us the area under curves having associated criterion, therefore calculating the cut-off values of β-hCG, urea, creatinine and AFI for diagnosing PROM were more than 9mg/dl, more than 3.4 mg/dl, more than 0.08 mg/dl and less than or equal to 7.2 cm respectively. And the sensitivity and specificity for the given markers were also calculated for diagnosing PROM and were 95% and 83.33% for β-hCG, 63.33% and 98.33% for urea, 86.67% and 48.33% for creatinine and 70% and 85% for AFI (figure 1, 2). Area under curves for all the markers have values of statistically significance of p <0.001.
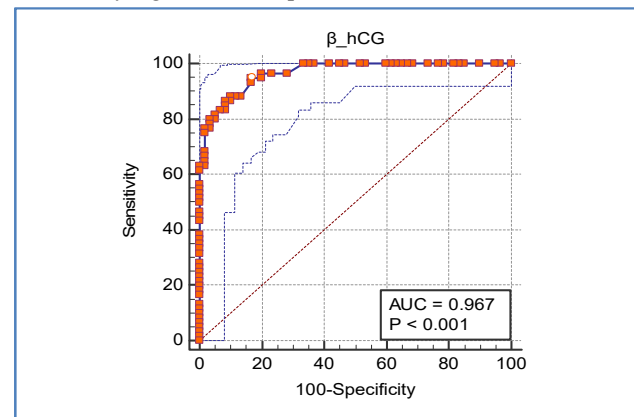

Figure 1: ROC curve to calculate the cut-off value of beta-hCG for the diagnosis of PROM
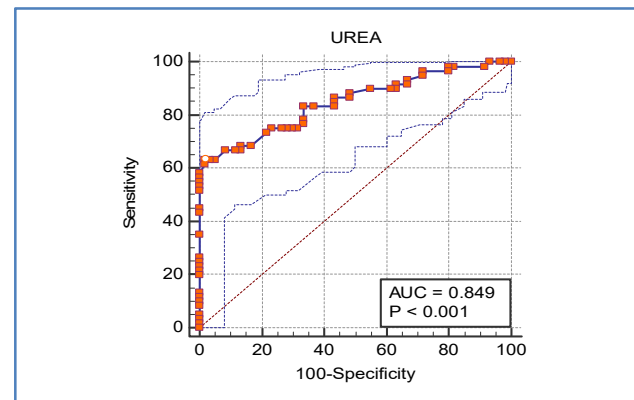

Figure 2: ROC curve to calculate the cut-off value of serum urea for the diagnosis of PROM

Discussion

The importance of correct diagnosis of PROM is useful for the obstetrician to manage pregnancy and its outcome and to avoid any complications with proper planning of time for termination of pregnancy.

Mean age in our study group of non-PROM and PROM patients were 26.92±3.39 and 26.32±3.00 years, similar in a study by Kuruoglu et al [12], they took mean maternal age 26.3 years in PROM group and 28.8 years in non-PROM patients. In our present study, PROM group, the majority of the women were primigravida, though it does not hold any significance in our study, which in even other studies like, Ismail El-Garhy et al [13] showed that there was no statistical significance.

In our study, the mean value β-hCG was 282 IU/l in PROM patients and 6.27mIU/ml in non-PROM patients, which is statistically significant. Similarly, in study by Marzieh Ghasemi et al [8] showed mean value of β-hCG, was 203.1±180.9 mIU/mL in the cases and 17.4±9.9 mIU/mL in the control. While Zinatossadat Bouzari et al [10] demarcated the values of β-hCG 39.5 IU/L which is quite less than our beta hCG value in PROM cases, which might be due to variations in amount of samples collect or amount of saline pushed for vaginal irrigations, an operative based error.

In our study, 60 participants of PROM group had mean vaginal urea levels of 10.80 mg/dl, and on comparison with the study done in 2017, by Begum et al [1] who did their research work on vaginal urea levels was higher in study group with cut-off values >6mg/dl to diagnose PROM cases, which is lesser than our calculated values. Also in another study done by H M Borg et al [14] observed that the mean vaginal fluid urea in confirmed, suspected and control groups were 20.3, 7.41, 2.6 mg/dl respectively. The sensitivity, specificity of vaginal fluid urea was 96%, 95% respectively, with cut-off values > 4 mg/dl.

In 2019, by Kuruoglu et al [12] vaginal creatinine was found to have 94.4% sensitivity, 93.3% specificity, 85% positive predictive value, and 97.7% negative predictive value in PROM confirmed cases. Therefore, mean creatinine values in the PROM and non-PROM groups were 0.39 mg/dl and 0.04 mg/dl, respectively which is lesser than our observation values of mean creatinine in PROM group which is 0.70 mg/dl. On further comparing our results, with a recently done research work in 2019, by Ruchika Garg et al [15] demarcated that vaginal creatinine had 74.4% sensitivity, 84.8% specificity, higher than our study's sensitivity but lower specificity. But a higher value of sensitivity than specificity like in our research work showed us that creatinine alongwith other markers is useful from differentiating PROM patients from non-PROM patients.

We calculated AFI among cases and control and located that most of PROM participants were borderline oligohydramnios (6.69±2.11) which was statistically significant. Kuruoglu et al [12] stated in their research that there were no statistically significance differences between mean amniotic fluid indices seen in their participants group. This was because, in their study cases were PROM, some with leaking P/V freshly began and some with control group, who were already having oligohydramnios.

The strengths of our study were that we used three parameters to diagnose PROM. As such there were no limitations associated with our study, except mild to moderate pain which patients experience while taking vaginal wash samples.

Conclusions

Detection of β-hCG, urea and creatinine in vaginal fluid to diagnose PROM has high sensitivity, specificity. It is a simple, reliable and rapid test. Introduction of this method into routine use is feasible and practical as there is no need for extra equipment, reagents, and affordable in country like India. Therefore, it is cost effective for PROM. They can be used as an adjunctive test in equivocal cases of PROM. Difference in sample sizes, inclusion criteria and the gestational age of studied patients might have resulted in the difference in the cut-off levels between the various studies. We suggest further studies can be taken up for determination of cut-off values of vaginal wash β-hCG, urea and creatinine for diagnosing rupture of membranes in pregnancy.

Conflict of interest: None. **Disclaimer:** Nil.

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