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Research Article

DIAGNOSIS OF PREMATURE RUPTURE OF MEMBRANES BY ESTIMATION OF PROLACTIN IN VAGINAL WASHING FLUID

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A B S T R A C T

Objective: Premature rupture of membranes is defined as the rupture of foetal membranes which may occur at any time before the onset of labour. The purpose of this study was to assess the reliability of cervico-vaginal fluid prolactin level for the diagnosis of premature rupture of membranes and to determine its diagnostic cut off value. **Method:** A total of 80 pregnant women were included, of which, 40 pregnant women between 20 to 41 weeks of gestation, complaining of leaking PV with amniotic fluid pooling +, nitrazine test +, and fern test +, were included in the study group and 40 pregnant women with no complaints were included in control group. All patients underwent USG for AFI calculation and vaginal washing fluid sampling for prolactin assay. **Results:** For PROM 34.35 µIU/ml (area under the curve (AUC) = 0.953) was the optimal cut-off value of vaginal prolactin, with a sensitivity of 92.5% and a specificity of 87.5%. It had PPV of 88.09%, NPV of 92.11% and accuracy of 90%. TheStandard Errorwas0.024. **Conclusion:** Vaginal washing fluid prolactin is a suitable marker for the diagnosis of PROM which can be used as an adjunctive test particularly in ambiguous cases of PROM.

INTRODUCTION

Premature rupture of membranes is defined as the rupture of foetal membranes minimum 1 hour before the onset of labour[1] and at any gestational age, even at 42 weeks[2, 3]. PROM, a complication of 2% to 25% of all deliveries[4], is a known important contributor to maternal and perinatal morbidity and perinatal mortality. It has been shown to cause 18-20% of perinatal mortality[5] and 21.4% of perinatal morbidity[6]. Average hospitalization period for term and preterm newborns with PROM is shown to be prolonged by 20% and 25% respectively[7]. Maternal complications occur in 13% to 60% of women with PROM in comparison with 1% prevalence of term and postpartum endometritis[8, 9].

The interval between PROM and birth[10](Latency) is known to be inversely related to gestational age at rupture.It is also related to a multitude of other factors, including number of foetuses[11], severity of oligohydramnios[12], myometrial thickness[13], and existence of maternal or obstetrical complications. The major cause of perinatal morbidity and mortality associated with PROM is prematurity[14]. Morbidities related to prematurity include respiratory distress syndrome, necrotizing enterocolitis, interventricular hemorrhage, cerebral palsy, sepsis, in utero umbilical cord compression, cord prolapse and foetal distress, foetal malpresentation, placental abruption, chorioamnionitis with subsequent endometritis and increased risk of operative delivery. Maternal sepsis, a rare but life-threatening complication, is reported in approx 1% of cases[14].

Failure to identify women with membrane rupture may result in failure to implement obstetric measures. Conversely, false diagnosis of membrane rupture can lead to inapt interventions such as hospitalization or induction of labour. Therefore, it is very important to establish a definite diagnosis of ruptured membranes in uncertain cases without delay.

Traditional diagnostic methods and tests have some limitations and cannot be applied to all women with 100% accuracy.The traditional minimally invasive gold standard for diagnosis of PROM relies on the following three clinical signs on sterile speculum examination:

1) Visual pooling of clear fluid in posterior fornix of the vagina or leakage of fluid from cervical os[15].

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2) Alkaline ph of cervico-vaginal discharge demonstrated by nitrazine paper[16].
3) Microscopic ferning of cervico-vaginal discharge[3, 4, 16].

The absence of a non-invasive 'gold standard' test for the diagnosis of membrane rupture has led to the investigators to seek for alternative diagnostic methods such as detection of some biochemical markers in vaginal fluid, which have high amniotic fluid concentration. Some of these markers include β-human chorionic gonadotropin (β-hcg)[17, 18, 19], prolactin[20, 21, 22], fetal fibronectin, α-fetoprotein (AFP)[22, 23], diamino-oxidase (DAO)[24] and insulin-like growth factor binding protein-1 (IGFBP-1)[25].

Thus, we hypothesized that vaginal fluid prolactin may be helpful in diagnosis of PROM.

MATERIAL AND METHOD

This study was conducted in the Department Of Obstetrics and Gynaecology, SMS Medical College, Jaipur, from June 2015 to November 2016 after approval from ethical committee. 40 pregnant women between 20 to 41 weeks of gestation with complaints of leaking PV with informed consent were included in the study group. Pregnant women with vaginal bleeding (either spontaneous or traumatic), regular uterine contractions, multiple pregnancy, use of vaginal drugs, intercourse in previous night, meconium in amniotic fluid, presence of foetal anomalies, intrauterine foetal death and suspicious PROM were not included in the study. All the women included in the study, underwent sterile speculum examination in lithotomy position to check for pooling of amniotic fluid in the posterior fornix of vagina, with and without valsalva maneuver. A cotton tip applicator was inserted in the posterior fornix of vagina to get a sample of vaginal fluid. It was then immediately transferred to nitrazine paper to check for ph of the fluid. A ph of more than 6.5 was considered as positive test. A sample of cervico-vaginal fluid was taken again and spread over a clean glass slide and allowed to dry. These slides were then examined under microscope (10x magnification) to check for ferning pattern. Thus, women who had positive pooling, nitrazine test and fern test were considered as study group (confirmed PROM group). Whereas 40 pregnant women, who were admitted to prenatal clinic for their regular prenatal control visit, without any complaints or complications and with pooling (-), nitrazine test (-) and fern test (-) were taken as control group.All these 80 pregnant women then underwent ultrasonographic examination for calculation of amniotic fluid index and vaginal washing fluid prolactin sampling.

Vaginal washing fluid prolactin sampling was done as follows: 5 ml of sterile saline solution was injected into the posterior vaginal fornix and 3 ml of this fluid was withdrawn with the same syringe. This sample was then centrifuged for 3 minutes at 1500 revolutions per minute. The supernatant was taken to S.M.S. Central Laboratory for quantitative measurement of prolactin level by electrochemoluminescence method using COBAS machine.

Continuous variables were summarized as mean and standard deviation whereas nominal/ categorical variables as proportions.Unpaired t-test and Mann Whitney test was used for analysis of continuous variables while chi square test was used for nominal/categorical variables. ROC curve was made to determine optimum cut-off value of cervico-vaginal fluid Prolactin level for determination of PROM.

RESULTS

The two groups of 40 women each were comparable with respect to age, literacy, socioeconomic status and occupation. The mean age of women in the study group was 23.20 ± 2.96 years and in the control group, it was 24.20 ± 2.95 years. Mean parity, in both study as well as control group, was approximately 0.4 (0.4 ± 0.81 in study group and 0.425 ± 0.75 in control group). Mean gravidity, in study and in control group, were 1.60 ± 1.464 and 1.53 ± 0.816, respectively. There was no statistically significant difference was observed between the study and control group with respect to mean age, mean parity, mean gravidity and mean apgar score of the neonates as shown in table 1.

Table 1 Descriptive data of the groups

	Study group (n=40)	**Control group (n=40)**	**P value**
Mean age (in yrs)	23.20 ± 2.96	24.20 ± 2.95	0.851
Mean parity	0.4 ± 0.81	0.425 ± 0.75	1.000
Mean gravidity	1.60±1.464	1.53± 0.816	0.841
Mean APGAR score	6.68 ± 0.764	6.90 ± 0.379	0.308

AFI was significantly lower in women with PROM, as campared to control group, shown in Table 2. AFI <5 was found in 70% of study group as compared to 10% of controls. Only one woman in the study group had AFI greater than 8.

Table 2 Amniotic fluid index

	Study group		**Control group**		**Total**	
AFI	No	%	No	%	No	%
<5	28	70	4	10	32	40
5 to 6	5	12.5	6	15	11	13.75
7 to 8	6	15	25	62.5	31	38.75
>8	1	2.5	5	12.5	6	7.5
Total	40	100	40	100	80	100

The mean vaginal prolactin level in the study group was 1457.77 ± 1310.85 µIU/ml (range 4255.20 – 10.30 µIU/ml), while in control group, it was 41.75 ±127.11 µIU/ml (range 800.3 – 9.40 µIU/ml). This shows that levels obtained for the study group were significantly higher than the levels obtained for the control group as shown in table 3.

Table 3 Vaginal washing fluid prolactin level among groups

	Study group	**Control group**	**P value**
Mean Prolactin Levels (µIU/ml)	1457.77±1310.85	41.75±127.11	<0.0001S
Max to Min	4255.20 – 10.30	800.3 – 9.40	

ROC curve analysis was performed to determine optimal cut-off values of significant variables (prolactin) detected between

the two groups (Figure 1). A 34.35 µIU/ml (area under the curve (AUC) = 0.953) optimal cut- off value of vaginal prolactin, with a sensitivity of 92.5% and a specificity of 87.5%, PPV of 88.09%, NPV of 92.11% and accuracy of 90%, was determined for PROM with SE 0.024. (True Positive = 37, True Negative = 35, False Positive =5, False Negative =3).

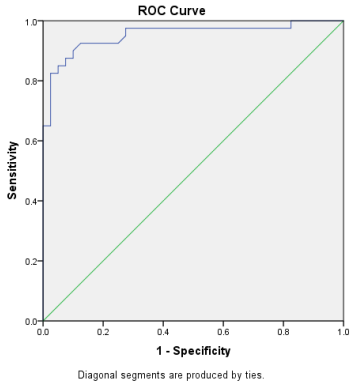

Figure 1 ROC curve

DISCUSSION

A prompt and accurate diagnosis of PROM is important for improved perinatal outcome and to minimize the serious outcomes. In majority of the pregnant women, diagnosis is made either on the basis of the clinical complaints which are not reliable or traditional methods such as visualization of amniotic fluid pooling on speculum examination, nitrazine paper test or ferning pattern. Except for the direct visualization of amniotic fluid spurting from the cervical os, all other traditional methods have their limitations.

Visualization of amniotic fluid pooling in the posterior fornix has a high false negative rate as non visualization of pooling does not exclude PROM. The reliability of nitrazine paper test is not good after 48 hoursof pooling due to build up of acidic vaginal pH. Similarly, fern test also has a high false positive rate because of the presence of cervical mucus which may interfere with amniotic fluid ferning pattern.

Prolactin, a 199-aminoacid single polypeptide chain, is encoded by a single gene located on short arm of chromosome 6. During pregnancy, it is produced by maternal hypophysis, fetal hypophysis and decidua[26]. In amniotic fluid, the prolactin level is approximately 5–10 times that in the maternal circulation[27], which is thought to be secreted by the decidua. The amniotic fluid prolactin level is between 1200 and 7000 ng/ml in the first 20 weeks of gestation, which then declines to about 350 ng/ml (10µIU/ml = 0.47 ng/ml) at term[28].

Various studies relating to PROM and vaginal washing fluid prolactin have been conducted so far.N. Karimanfound thatthe mean concentration of vaginal fluid prolactin level in PROM group was 851.22 ± 425.74 µIU/ml (range 5.00-5551), which was significantly higher than values obtained for control group i.e., 8.20±0.67µIU/ml (range 4.00-24.00). From ROC curve, 9.50µIU/ml was set as a cut-off value for prolactin by him[29].Shahin and Raslanshowed that vaginal fluid concentration of prolactin in PROM group was 28.48±10.54 µIU/ml as compared to control group i.e., 16.98±7.69 µIU/ml and a cut-off value of 20.2 µIU/ml was proposed for PRL[30]. Buyukbayrak (2004) found that the mean value of prolactin in vaginal washing fluid as 616.59 µIU/ml in the confirmed PROM group, 23.98 µIU/ml in the suspected but unconfirmed PROM group and 10 µIU/ml in the control group ($p < 0.0001$) and diagnostic cut-off value of vaginal washing fluid prolactin for diagnosis of PROM was found to be 30 µIU/ml[31]. However, Phocas(1989) concluded that in PROM, vaginal fluid PRL levels were significantly higher (2-10 fold) than the paired maternal serum PRL and ranged from 130 - 2315 ng/ml. In contrast, vaginal PRL and urine PRL concentration in pregnancies without PROM were very low or undetectable[19].

Koninckx and associates (1981)collected samples of urine, blood and vaginal washing fluid before and after artificial rupture of membranes and found that there were no significant differences in the prolactin concentrations of urine and plasma samples before and after membrane rupture, but the concentration in vaginal washing fluid was significantly different before and after membrane rupture (<3 mU/ml before rupture, 6–70 mU/ml after rupture)[20].The findings of our study are similar to these studies.

In contrast, Huber assayed the amount of PRL, AFP and hPL in vaginal washing fluid. Despite the higher concentration levels of the three markers in PROM group, he concluded that, the measurement of these proteins in vaginal fluid could not be a suitable clinical test for the diagnosis of PROM, due to the presence of considerable overlap between the groups and a high rate of false positives[23].

CONCLUSION

Vaginal washing fluid prolactin is a suitable marker for the diagnosis of premature rupture of membranes. It can be used as an adjunctive test particularly in ambiguous cases of PROM where the diagnosis is doubtful, so that timely proper intervention may be taken to improve maternal and neonatal outcome. It has a diagnostic cut-off value of 34.35 µIU/ml with a high sensitivity of 92.5%, specificity of 87.5%, a negative predictive value of 92.11%, positive predictive value of 88.09% and accuracy of 90%.

However, the limitation of our study was that electrochemoluminescence assay, which was used to quantitate vaginal washing fluid prolactin level in our study, is often a batch assay that takes a long time to perform. It is also a complex test to perform and also not routinely available in most laboratories. An ideal test should be easily available to the women.

Declarations

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