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Original Research Article

Vaginal fluid creatinine as a promising alternative to existing diagnostic test for premature rupture of membranes (PROM)

Neetu Singh[1], Rashmi Verma[1], Devyani Misra[1], Manish Raj Kulshrestha[2], Vandana Tiwari[2], Rupita Kulshrestha[1]*

[1]Dept. of Obstetrics and Gynaecology, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, Uttar Pradesh, India
[2]Dept. of Biochemistry, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

Abstract

Background: Premature rupture of membranes (PROM) is associated with complications such as preterm birth, chorioamnionitis etc. The incidence of PROM at term gestation is about 10% and that of preterm premature rupture of membrane is 3% globally. Normal vaginal fluid does not contain creatinine so only when the membranes ruptures creatinine is detected in vagina. So, the present study aims to detect the correlation between vaginal fluid creatinine levels and PROM.

Materials and Methods: The women admitted between 28 to 37 weeks of pregnancy fulfilling inclusion and exclusion criteria were enrolled in our study after their informed consent. They were categorised as group 1 (cases) and group 2 (controls) (n=140 each, on the basis of history of leaking per vaginum). They were investigated by doing relevant investigations and clinical examination. Vaginal fluid was collected & sent to Department of Biochemistry for creatinine estimation. All patients participating in this study were followed till delivery to record maternal and fetal outcomes.

Results: Data so gathered was analysed on IBM SPSS 21.0 software. Parametres such as age, parity, BMI, gestation age, occupation and social background did not show any statistical significance among cases and controls. Cases had significantly higher creatinine levels in vaginal fluid & lower Amniotic fluid index (AFI) as those in controls. Cases had higher risk of chorioamnionitis, prolonged hospital stay & surgical site infections. Neonatal Intensive care unit (NICU) admission and still births were significantly higher in cases than controls.

Conclusion: PROM is associated with increased maternal and neonatal complications. Vaginal fluid creatinine estimation might prove cost effective and feasible method as there is no need for extra equipment & reagent for detection of PROM.

Keywords: Vaginal fluid creatinine, Premature rupture of membranes (PROM), Chorioamnionitis, Surgical site infections, Still births, NICU.

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1. Introduction

Premature rupture of membranes (PROM) refers "to the spontaneous rupture of the amniotic sac prior to the start of labor. When PROM occurs before 37 weeks of gestation, it is classified as preterm PROM, which can lead to complications such as preterm birth and chorioamnionitis".[1] The occurrence of PROM at full term is approximately 10%, whereas the global incidence of preterm PROM is around 3%.[2-4]

The risk of infection for both the mother and the fetus increases as the time duration between the rupture of membranes and the onset of labor time increases. About 3 to 15% of mothers with PROM get infections, while around 5% of all babies whose mothers have PROM get infections. This rate goes up to 15-20% if the mother develops chorioamnionitis.[5,6]

Detecting membrane rupture early and accurately is primal for providing appropriate care based on the baby's gestational age, which helps improve outcomes and reduce complications. Testing for creatinine in vaginal fluid can help diagnose PROM (as fetal urine), a main constituent of amniotic fluid (AF) in the latter half of pregnancy duration, contains creatinine.[1] Since there isn't a definitive diagnostic method, various approaches using biochemical markers have been developed. Normally, the vagina does not contain creatinine, so its presence indicates membrane rupture.[4] The

*Corresponding author: Rupita Kulshrestha
Email: rupita.kulshrestha@gmail

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level of creatinine in AF rises significantly between the 20th and 32nd weeks of pregnancy, reaching levels 2-4 times higher than in the mother's blood.[5]

2. Materials and Methods

2.1. Study design and setting

This prospective observational study with non-probability sampling was conducted at the departments of obstetrics & gynaecology and biochemistry of our institution. An informed written consent was also obtained from all participants. Sample size was calculated using the formula, $n= (Z1-\alpha/2)2 \text{ X } p(1-p)/d^2$. Prevalence of cases of premature rupture of membrane was considered 10%. 140 subjects were enrolled in the each group.

The antenatal patients enrolled between ≥28 weeks to ≤37 weeks of gestation with singleton live intrauterine pregnancy and divided in two groups. Group 1 (PROM/case) consisted of 140 pregnant women who had a history of vaginal leaking, confirmed by a speculum examination revealing amniotic fluid pooling in the posterior fornix or fluid emerging from the cervix. Group 2 (non-PROM/control) also comprised 140 pregnant women, but these women had no history of vaginal leaking and their speculum examinations were negative for amniotic fluid pooling in the posterior fornix or fluid coming out of the cervix.

2.2. Inclusion criteria

1. Women with a single pregnancy at ≥28 and ≤37 weeks of gestation (determined by last menstrual period or first-trimester ultrasound) who have a history of vaginal leaking.
2. Patients willing for the participation in our research study.

2.3. Exclusion criteria

1. Pregnant women experiencing vaginal spotting or bleeding.
2. Pregnant women with vaginal infection, use of vaginal drugs, sexual activity in last 24 hours.
3. Pregnant women present with regular uterine contractions.
4. Pregnancy with hypertension or preeclampsia, liver or kidney diseases, heart disease, thyroid disorder, chronic urinary tract infection, multiple pregnancies, infertility treated pregnancy.
5. Pregnant women with uterine anomalies, intrauterine fetal demise, congenital malformations.
6. Patient not willing for participation in the research study.

2.4. Study protocol

A comprehensive history, physical examination, and routine investigations were conducted, followed by a sterile speculum examination to check for fluid emanating from the cervix or pooling in the posterior fornix. Subsequently, a sample of vaginal fluid was collected.

2.5. Sterile per speculum examination

During the speculum examination, 5 cc of saline was injected into the vagina, and 3 cc was then retrieved into a tube using the same injector. Then sample was promptly sent to the laboratory of biochemistry for centrifugation. Further, creatinine was measured by Modified Jaffe's method based on Backmen Coulter AU 480 autoanalyser using commercial kits..

Transabdominal sonography for fetal wellbeing was done for detailed obstetrical finding including GA, AFI, Fetal viability etc. Blood investigations viz. LFT, random blood sugar, RFT, thyroid profile, urine routine and microscopy, urine culture-sensitivity and vaginal swab culture and sensitivity were also performed. All patients participating in this study were followed till delivery for assessing maternal and neonatal outcomes.

3. Results

In the group 1 (PROM group) mean age of patients was 27.21±6.91years while group 2 (non-PROM group) it was 28.24±5.87 years which was comparable (p=0.124). Major age group was 26-30 years, parity was P=0, GA was 35-37 weeks in both the groups. All of which were not statistically significant (p>0.005). Mean BMI in group1 was 24.53±5.91 kg/m2, while in group 2 was 25.27±6.87 kg/m^2 that was again statistically not significant. Out of 280 patients, 173(61.7%) were rural, 107(38.2%) were urban. Also, 237(84.7%) were house wives and remaining 43(15.3%) were occupational. Parameters which showed statistically significant difference were AFI and vaginal fluid creatinine levels (p<0.005). Mean AFI in group 1 was 7.02±1.62 cm while in group 2 was 12.12±2.14cm (p= 0.002). The vaginal fluid creatinine levels in group 1 was 1.26±0.55 mg/dl while in group 2 was 0.005±0.04 mg/dl (p< 0.001). Detailed socio-demographic and obstetric observations have been shown in **Table 1**.

Study of maternal outcome among both groups showed that complications like chorioamnionitis, emergency LSCS, PPH, postpartum febrile illness, surgical site infection were statistically more common in PROM group vs non-PROM group. Prolonged hospital stay was also more on PROM group as compared to non-PROM group. Detailed observations are shown in **Table 2**. For fetal outcome, significant difference was observed in still births and NICU admissions in PROM group as compared to non-PROM group (p<0.001) as shown in **Table 3**.

After documenting the observations, ROC values were calculated as presented in **Table 4**. The sensitivity was 97.6%, specificity was 80.1%, positive predictive value (PPV) was 82.6%, negative predictive value (NPV) was

90.6%, and accuracy was 90.6%. **Table 5** displays the overall performance of vaginal fluid creatinine levels as an indicator for PROM. ROC curve analysis determined the optimal cut-off value for vaginal fluid creatinine, with AUC being 0.992 for creatinine.

Table 1: Socio-demographic, obstetric variables of study participants

Variable	Gp 1 (n=140)	Gp 2 (n=140)	Total N (%)	p-value
Age gp				
≤20 years	4 (2.85%)	3 (2.14%)	7 (2.5%)	
21 to 25 years	43 (30.71%)	38 (27.14%)	81(28.9%)	0.211
26 to 30 years	72 (51.42%)	81 (57.85%)	153(54.6%)	0.222
≥ 30 years	21 (15%)	18 (12.85%)	39(13.9%)	0.154
Mean age ±SD	27.21±6.91	28.24±5.87	-	0.124
Parity				
P – 0	89 (63.57%)	81 (57.85%)	170(60.7%)	
P – 1	23 (16.42%)	29 (20.71%)	52(18.5%)	
P – 2	19 (13.57%)	22 (15.71%)	41(14.6%)	
≥ P – 3	9 (6.42%)	8 (5.71%)	17(6.1%)	
mean BMI	24.53±5.91	25.27±6.87	-	0.241
GA				
28 to 31 weeks	3 (2.14%)	5 (3.57%)	8(2.8%)	
32 to 34 weeks	53 (37.85%)	51 (36.42%)	104(37.1%)	
35 to 37 weeks	84 (60.00%)	84 (60.00%)	168(60%)	
Mean±SD	34.6±4.31	33.2±3.87	-	0.521
Occupation (working)	24 (17.14%)	19 (13.57%)	43(15.3%)	
Housewife) (n)	116 (82.85%)	121 (86.42%)	237(84.7%)	
Rural (n)	84 (60%)	89 (63.57%)	173(61.7%)	
Urban (n)	56 (40%)	51 (36.42%)	107(38.2%)	
Amniotic fluid index (AFI) Mean±SD (cm)	7.02±1.62	12.12±2.14	-	0.002
Vaginal fluid Creatinine level (Mean±SD)				
28 to 31 weeks	0.11±0.08	0.003±0.001	-	<0.001
32 to 34 weeks	0.89±0.12	0.003±0.001	-	<0.001
35 to 37 weeks	0.70±0.08	0.002±0.001	-	<0.001
Vaginal fluid creatinine (mg/dl)	1.26±0.55	0.005±0.04	-	<0.001

Table 2: Performance of vaginal fluid creatinine level in prediction of PROM among study cases

Parameter	Screening test positive	Screening test negative	Total
PROM Group	136	4	140
Non-PROM group	8	132	140
Total	144	136	280

Table 3: Performance indices of vaginal fluid creatinine level in prediction of PROM among study cases

Parameter	Creatinine
Cut off	0.281
AUC	0.992
Sensitivity	97.6
Specificity	80.1
PPV	82.6
NPV	98.7
Accuracy	90.6
P value	<0.001

Table 4: Maternal outcome in PROM (group 1) and non PROM (group 2) patients

Maternal Outcomes	Group 1 (n=140)	Group 2 (n=140)	P value
Chorioamnionitis	5 (3%)	0	0.001
Vaginal delivery	86 (61.4%)	116 (82.85%)	0.003
Emergency LSCS	54 (38.5%)	24 (17.14%)	0.001
Post partum haemmorage	13 (9.2%)	5 (3.5%)	0.002
Post partum febrile illness	18 (12.8%)	6 (4.28%)	0.002
Surgical site infection	15 (10.7%)	2 (1.4%)	0.001
Prolonged hospital stay (>15 days)	30 (21.4%)	2 (1.4%)	<0.001

Table 5: Fetal outcome in PROM (group 1) and non-PROM (group 2) patients

Fetal Outcomes	Babies of group 1 (n=140) n (%)	Babies of group 2 (n=140) n (%)	P value
Alive and Healthy	65 (46.4%)	112 (80%)	
Still birth	15 (10.7%)	1 (0.71%)	<0.001
NICU admission	60 (42.85%)	27 (19.28%)	<0.001

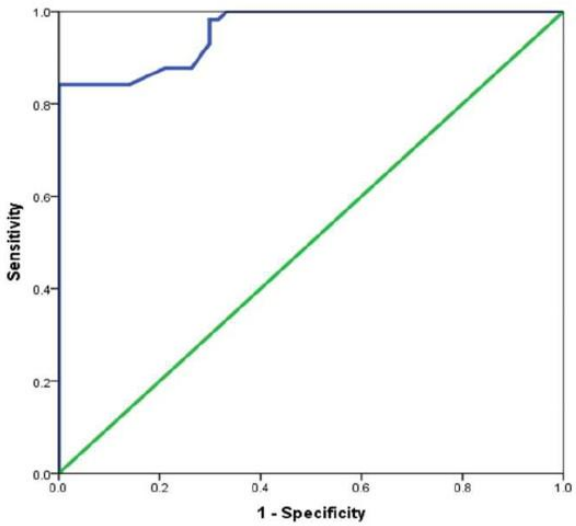

Figure 1: ROC curve showing performance of vaginal fluid creatinine in prediction of PROM in studied patients

4. Discussion

We determined the level of vaginal fluid creatinine in detection of premature rupture of membranes along with its value in different gestational age group and its effect on pregnancy outcome with the duration of leaking per vagina. The study was performed on 280 pregnant women between ≥28 to ≤37 weeks of gestation, which was divided into control and cases.

In our study we observed that the maximum number of subjects were between 21 to 30 years. The age of both groups was not significant statistically. Kariman et al did a study and showed similar mean age distribution 26.4±2.45 years in confirm PROM group and 25.06±1.34 years in controls,[7] which was not statistically significant (as in our study).

In our study mean BMI (Kg/m2) of cases was 24.53±5.91 and controls was 25.27±6.87 and the p value was 0.241, which was statistically not significant. Participants of primigravida in Group 1 were 89 (63.57%) and in Group 2 were 51 (36.42%). Similarly, participants of Multi Gravida in Group 1 were 51 (36.42%) and Group 2 were 59 (42.14%). Similar result showed by Kuruoglu YS et al[8] and Jasmina Begum et al[9] study. Jasmina Begum[9] reported no statistically significant differences in terms of maternal age and gestational age at the time of sampling, which is consistent with our findings. Oligohydramnios may not be observed in individuals with confirmed PROM, possibly because drainage can become intermittent or cease altogether after the presenting part descends and acts as a plug, preventing further fluid loss. However, Jasmina Begum[9] discovered that a decrease in the four-quadrant AFI to ≤ 7 cm is not effective in diagnosing membrane rupture based on history, fluid visualization during a speculum examination, and a positive nitrazine paper test.

Their investigation found that ultrasonography assessment of AFI has low diagnostic accuracy for membrane rupture. But in present study, mean AFI levels in both cases and controls were found to be statistically significant with values mean AFI in Group1 was 7.02±1.62 and Group 2 was 12.12±2.14 (p<0.005).

Here, mean vaginal fluid creatinine level were seen to be increasing with gestational age in group 1, while no changes of vaginal fluid creatinine level with gestational age seen in group 2. This was in agreement to study by Kuruoglu YS et al.[8] The mean creatinine level in cases was 1.26±0.55 and for the controls was 0.005±0.04. The p value for it was statistically significant (p < 0.001). These findings are also in agreement of other studies done by Kuruoglu YS et al.[8] and Ghasemi et al.[10]

According to the ROC curve analysis, the cutoff point for creatinine was determined to be 0.25 mg/dL, yielding 74.6% sensitivity and 85% specificity for diagnosing PROM. Li Hy et al. conducted a study investigating the levels of hCG, AFP, and creatinine in vaginal fluid among PROM cases. Their study concluded that evaluating hCG, AFP, and creatinine in vaginal flushing fluid proved beneficial for diagnosing PROM.[11] However, the assessment of creatinine in vaginal fluid was found to be more cost-effective and simpler compared to estimating hCG and AFP in the same fluid. The optimal cutoff concentrations for creatinine in vaginal washing fluid were established through ROC curve analysis, with creatinine exhibiting an area under the curve of 0.992.

Fatemeh Malchi et al. conducted a meta-analysis to evaluate the diagnostic accuracy of vaginal urea and creatinine levels in identifying PROM.[12] Analysis encompassed eleven trials with a total of 1324 participants. Their findings revealed that the average urea and creatinine levels in patients were notably higher compared to those in controls (12.63 vs. 0.31). This comprehensive review concluded that the mean urea and creatinine levels in the case group were significantly increased compared to those in the control group, with creatinine demonstrating increased sensitivity and specificity than urea in diagnosing PROM.

Leila Sekhavat et al. conducted a study evaluating the utility of creatinine determination in vaginal washing fluid as a diagnostic marker for PROM.[13]. They concluded that this method is simple and fast for measuring vaginal fluid creatinine levels in diagnosing premature rupture of membranes. Their observations revealed sensitivity, specificity, PPV and NPV, and accuracy of vaginal fluid creatinine concentration at 98.7%, 100%, 100%, 98.8%, and 87.1%, respectively, with a cutoff value of 0.14 mg/dl for detecting PROM.[13]

Abayomi Ibukun Alao et al. demonstrated the high effectiveness of vaginal fluid urea and creatinine as diagnostic tests for PROM.[14] They reported sensitivity, specificity, NPV, and PPV of vaginal fluid urea and creatinine at 94%, 82%, 93.18%, and 83.93%, respectively, as well as 98%, 90%, 97.82%, and 90.74%,[14] which align with similar outcomes in the present study.

Adebunmi O. Olarinoye et al. evaluated the reliability of nitrazine, urea, and creatinine in diagnosing PROM and found that the nitrazine strip, which detects pH levels, was the most reliable among the three, followed by creatinine and urea.[15]

Kafali and Oksuzler investigated the role of vaginal fluid creatinine in PROM cases and noted a significant difference in mean vaginal fluid creatinine levels between PROM cases and controls, with 100% sensitivity, specificity, PPV, and NPV in PROM diagnosis. They concluded that detecting urea and creatinine in vaginal washing fluid for PROM diagnosis is a straightforward, reliable, and swift test.[16]

Zanjini et al investigated the vaginal fluid creatinine in 60 patients who presented with PROM. The confirmed group had considerably higher creatinine levels ($p < 0.001$). The optimum cut-off value was 0.5 mg/dL, which provided 96.7% (sensitivity) and 100% (specificity).[17]

As far as feto-maternal outcome was concerned, 54 (38.5%) patients underwent emergency Cesarean Section among cases of PROM. Maternal morbidity in form of chorioamnionitis, PPH, wound infection, postpartum fever, prolonged hospital stay were more often observed in PROM cases. Patricia Berg et al. also studied the fetal outcome in clinical chorioamnionitis patients and reported that babies of diagnosed PROM and chorioamnionitis had more fetal tachycardia, raised maternal CRP levels, asphyxia related complications.[18] We observed that babies of PROM cases had more NICU admission and more number of still births in comparison to controls.

5. Conclusion

An appropriate and correct diagnosis of PROM is important for a successful pregnancy. This study reveals that PROM is a common pregnancy issue that causes increased maternal and neonatal difficulties such as surgical procedures, neonatal morbidity, and mortality. Detection of vaginal fluid creatinine to identify PROM is a simple, quick, and reliable test with good sensitivity, specificity, PPV, NPV, and overall accuracy. Diagnosis of PROM is challenging with existing procedures in equivocal cases, thus incorporating this method into everyday use is feasible and practical because there is no need for additional equipment or reagents. It may be made available in low-resource communities and is cost-effective for detecting PROM. Additionally, investigations with larger sample size might aid in identifying the greater application of this marker in the diagnosis of PROM.

6. Source of Funding

None.

7. Conflict of Interest

None.

8. Ethical Committee Approval

Ethical approval was taken from Institutional Ethics Committee (IEC- 120/20) of the institution.

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