Original Article

Raised Vaginal Fluid Fibronectin Level Indicates Premature Rupture of Membrane

Shamima Rahman[1], Samsad Jahan[2], Samira Humaira Habib[3], Nasrin Sultana[1], Mst Nigar Sultana[1], Debashish Paul[4], Amrita Bhowmik[4], Khodeja Begum[5]

Abstract

Background: *Premature rupture of membrane (PROM) is one of the common complications of pregnancy that has major impact on fetal and neonatal outcome. It is the commonest clinical event where a normal pregnancy becomes suddenly a high-risk one for mother and fetus or neonate.* ***Objective:*** *The study was undertaken to investigate whether raised fibronectin level in vaginal fluid may indicate premature rupture of membrane.* ***Materials and Methods:*** *This cross sectional study was conducted in the department of Obstetrics and Gynecology in Sir Salimullah Medical College & Mitford Hospital, Dhaka during the period of January 2006 to December 2007. A total of 114 pregnant women with gestational age 28th week up to 40th week were included. Sixty were PROM (Group I) and 54 were non-PROM (Group II) subjects. Fibronectin in vaginal fluid was measured by an immunochemical reaction by nephelometer. Statistical analysis was done by SPSS version 10.0.* ***Results:*** *The PROM patients had significantly higher concentration of fibronectin (225.77 ± 115.18 ng/mL) compared to that in non-PROM subjects (8.04 ± 16.17 ng/mL) ($p < 0.001$).* ***Conclusion:*** *It can be concluded that in cases of unequivocal rupture or intactness of the membranes, the result of the fibronectin test corresponds well with the clinical situation. So fibronectin is a sensitive test for detection of amniotic fluid in the vagina.*

Key words: *Vaginal fluid, Fibronectin, Rupture of membrane*

J Enam Med Col 2012; 2(2): 74-79

Introduction

Premature rupture of membrane (PROM) is one of the common complications of pregnancy that has major impact on fetal and neonatal outcome. PROM is the commonest clinical event where a normal traditional pregnancy becomes suddenly a high-risk one for mother and fetus or neonate.[1] The reported incidence of premature rupture of the membranes averages from 6% to 10% and about 20% of these cases occur before 37 weeks of gestation. Although preterm premature rupture of membranes complicate only 1% to 2% of all pregnancies, it is associated with 40% of preterm deliveries and can result in significant perinatal morbidity and mortality.[2] In PROM patients perinatal morbidity was 8% and usual causes of perinatal death are neonatal sepsis, severe asphyxia and respiratory distress syndrome.

Maternal morbidity following PROM is quite high. Increase in operative delivery rate increases postpartum infection rate up to 20 fold. PROM patients also suffer from endometritis and abruptio placente, and in these patients wound infection

1. *Junior Consultant, Department of Gynaecology and Obstetrics, Bangabandhu Sheikh Mujib Medical University, Dhaka*
2. *Associate Professor, Department of Gynaecology and Obstetrics, Ibrahim Medical College, Dhaka*
3. *Senior Research Officer, Health Economics Unit, Bangladesh Diabetic Somity, Dhaka*
4. *Research Fellow, Biomedical Research Group (BMRG), BIRDEM, Dhaka*
5. *Consultant, Department of Gynaecology, National Institute of Cancer Research and Hospital (NICRH), Dhaka*

Correspondence *Samsad Jahan, Email: shelly_birdem@yahoo.com, Phone: 01817049292*

occurs markedly at higher rate than in non-PROM patients.[3] So it is obvious that PROM is one of the obstetric conditions which lead to significant infant mortality and morbidity and maternal ill health.

Early diagnosis of PROM has a great impact on maternal and child health. Diagnosis of PROM requires a thorough history, physical examination and selected laboratory studies.[4] Commonly used methods for detection of amniotic fluid in the vagina include pooling, ferning, nitrazine paper test, ultrasound measurement of amniotic sac dimensions.[5] This study was undertaken to investigate whether raised fibronectin level in vaginal fluid may indicate premature rupture of membrane, to measure the concentrations of fibronectin in vaginal fluid of PROM and non-PROM patients and to assess the performance of fibronectin test for diagnosis of PROM by sensitivity, specificity, positive predictive value, negative predictive value and accuracy test.

Materials and Methods

This cross sectional study was conducted in the department of Obstetrics and Gynecology in Sir Salimullah Medical College & Mitford Hospital, Dhaka from January, 2006 to December, 2007. A total of 114 pregnant ladies with gestational age 28th week up to 40th week were included. Sixty were PROM (Group I) and 54 were non-PROM (Group II) patients. Both primipara and multipara patients with gestational age from 28th week up to 40th week pregnancy with history of spontaneous rupture of membrane within 72 hours and positive per speculum test for pooling were included in Group I. Both primipara and multipara women with gestational age from 28th week up to 40th week pregnancy with no history of spontaneous rupture of membrane and negative per speculum test for pooling were included in Group II. Verbal and written consent to participate in the study was taken from the patients. Patients with antepartum hemorrhage, induced or accidental rupture of membrane, presence of any blood on speculum examination or having sexual intercourse during last 24 hours were excluded from the study. Purposive (non probability) sampling was applied here for the convenience. The researcher interviewed the respondents according to their convenience after fulfillment of study criteria.

Confirmation of PROM

In this study, positive result of both nitrazine test and fern test (double positive) was taken as the gold standard for confirmation of diagnosis of PROM.

Specimen collection and preservation

For Group I patients

Patients were asked to lie in lithotomy position. After maintaining all aseptic precautions, a sterile speculum was introduced into patient's vagina. A sterile cotton tipped swab was used to collect fluid from the vagina and applied it to nitrazine paper. Nitrazine paper turned blue demonstrating an alkaline pH (7.0–7.25) if amniotic fluid was present in vaginal secretion. Another drop of fluid was placed on a slide and allowed to dry in air and was examined under low power (10×) microscope for fern pattern due to crystallization of sodium chloride derived from amniotic fluid.

Then sterile cotton tipped swab was inserted into posterior fornix of vagina, gently rotated across posterior fornix and around ectocervix for at least 10 seconds, and then extracted in 750 microliter of phosphate buffer. Specimens were stored at –20 degree centigrade.

For Group II patients

Only vaginal swab was collected for fibronectin concentration.

Principles of fibronectin measurement[6]

Fibronectin is measured by an immunochemical reaction by nephelometer. Fibronectin in the amniotic fluid sample form immune complexes with specific antibodies. These complexes scatter a beam of light passing through the sample. The intensity of the scattered light is proportional to the concentration of the relevant protein in the sample. The result is evaluated by comparison with a standard of known concentration. Taking the standard from a number of similar international studies conducted in Germany, Italy, Belgium and US, the fibronectin test was considered positive at the concentration of 50 ng/mL.

Ethical consideration

Before starting the research work permission was taken from the hospital authorities. All women enrolled in the study were explained about the nature

and purpose of the study and only those who gave written consent were included in the study.

Data collection

A preset self-administered questionnaire was used for data collection. Socio-demographic (age, occupation, monthly income), etiological (vaginal infection, urinary tract infection, antenatal care), present pregnancy related (parity, gestational age, watery discharge per vagina), past obstetric (history of PROM in previous pregnancy, history of MR, history of D & C) and diagnostic (nitrazine paper test, fern test, fibronectin test) variables were included. The raw data were edited properly.

Statistical analysis

All edited data were consolidated, processed statistically and analyzed with SPSS (Statistical Program for Social Science) Version 10.0. Statistical analyses were done by Unpaired Student's t test, Chi-square Test, Sensitivity, Specificity, Positive predictive value, Negative predictive value and Accuracy test.

Results

One hundred and fourteen pregnant patients with gestational age of 28^{th} to 40^{th} weeks who attended the outdoor department or were admitted in the hospital were included in the study. For Group I (PROM), 60 pregnant patients presenting with the clinical history of PROM within 72 hours and positive per speculum examination for pooling were selected. Then they underwent nitrazine paper test and fern test. In this study, positive result of both nitrazine test and fern test (double positive) was taken as the gold standard for confirmation of diagnosis of PROM. Of the 60 patients, 47 were found double positive in the test.

For Group II, 54 pregnant patients presenting with no history of PROM and negative per speculum examination for pooling were selected. Then both groups underwent fibronectin test. The findings of the study are presented below.

Age distribution of the subjects

Table I compares the age distribution between groups. In Group I 36.7% subjects were between 31–35 years, 35% between 26–30 years, 20% between 21–25 years, 5% were of 20 or below 20 years and 3.3% above 35 years of age. In the Group II nearly half (46.3%) of the subjects ranged from 26–30 years followed by 27.8% between 31–35 years, 22.2% between 21–25 years and 3.7% were of 20 or below 20 years. The groups were almost identical with respect to age (28.87 ± 4.15 vs 27.87 ± 3.82 years, p = 0.187).

Table I: Comparison of age between Group-I and Group-II (N = 114)

Age (yrs)	Groups		p value
	Group I (n = 60)	Group II (n = 54)	
<20	3 (5.0)	2 (3.7)	
21–25	12 (20.0)	12 (22.2)	
26–30	21 (35.0)	25 (46.3)	
31–35	22 (36.7)	15 (27.8)	
>35	2 (3.3)	0 (0.0)	
Mean ± SD	28.87 ± 4.15	27.87 ± 3.82	>0.05

Figures in the parentheses denote corresponding percentage. Data were analyzed using Student's t test and level of significance was 0.05.

Occupation of the subjects

About 57% of the Group I and 56% of the Group II were housewives. The service-holders formed the second majority in both the groups (28.3% in Group I and 29.6% in Group II) followed by day laborers (11.7% in Group I and 3.7% in Group II).

Income distribution of the subjects

Income distribution shows that 30% subjects of the Group I had monthly family income of Taka 5000 or below, 48.3% between Taka 5,000–10,000, 20% between Taka 10,000–15,000 and very few had Taka 15,000 or above. In Group II, majority (92.5%) had income of Taka 5,000–15,000 (44.4% between 5000–10,000 and 48.1% between 10,000–15,000). Very few subjects had income below 5000 or above 15000 taka.

Distribution of subjects by parity

Distribution of subjects by parity demonstrates that majority of the Group I (81.7%) and Group II (61.1%) was multipara. Multiparity was found to be

significantly associated with premature rupture of membrane (Table II).

Table II: Distribution of parity between groups (N = 114)

Parity	Groups		p value
	Group I (n = 60)	Group II (n = 54)	
Multipara	49 (81.7)	33 (61.1)	<0.05
Primipara	11 (18.3)	21 (38.9)	

Figures in the parentheses denote corresponding percentage. Data were analysed using Chi-square (χ^2) test and level of significance was 0.05.

Gestational age

Data pertaining to gestational age shows that 57% subjects of the Group I had preterm PROM and the 43% had term PROM.

Pattern of antenatal care

Table III shows that 48.3% subjects of the Group I did not receive any antenatal care (ANC) and only 28.3% received ANC regularly. In Group II, majority (83.3%) had the history of receiving regular antenatal care indicating that PROM is associated with no or irregular antenatal care ($p < 0.001$).

Table III: Distribution of subjects by antenatal care (N = 114)

Antenatal care	Groups		p value
	Group I (n = 60)	Group II (n = 54)	
None	29 (48.3)	1 (1.9)	
Regular	17 (28.3)	45 (83.3)	< 0.001
Irregular	14 (23.3)	8 (14.8)	

Figures in the parentheses indicate corresponding percentage. Chi-square (χ^2) test was done to analyse the data and level of significance was 0.05.

Infections during current pregnancy

Diseases acquired during current pregnancy demonstrate that approximately 36.7% subjects of the Group I had history of vaginal infection whereas it was 14.8% in Group II ($p = <0.001$). The incidence of urinary tract infection was found to be significantly higher in Group I (50.0%) than that in the Group II (24.1%) ($p = 0.004$).

Table IV: History of infections during current pregnancy (N = 114)

Infections during current pregnancy	Groups		p value
	Group I (n = 60)	Group II (n = 54)	
Vaginal infection	22 (36.7)	8 (14.8)	< 0.001
Urinary tract infection	30 (50.0)	13 (24.1)	< 0.01

Figures in the parentheses denote corresponding percentage. Data were analysed using Chi-square (χ^2) test and level of significance was 0.05.

Past obstetric history

Table V shows the association of PROM in previous pregnancy with that in current pregnancy. The Group I had a significantly higher frequency of PROM in previous pregnancy (31.7%) compared to Group II (1.9%) ($p = <0.001$). The past history of MR was 33.3% in Group I and 14.8% in Group II ($p = 0.022$). Regarding D & C there was no difference between the groups ($p = 0.138$).

Table V: Comparison of past obstetric history between groups (N = 114)

Past obstetric history	Groups		p value
	Group I (n = 60)	Group II (n= 54)	
PROM in previous pregnancy	19 (31.7)	1 (1.9)	< 0.001
Past history of MR	20 (33.3)	8 (14.8)	< 0.05
Past history of D & C	8 (13.3)	2 (3.7)	> 0.05

Figures in the parentheses denote corresponding percentage. Data were analysed using Chi-square (χ^2) test with continuity correction where needed.

Fibronectin concentration between groups

Table VI shows the comparison of fibronectin concentrations between groups. The Group I had significantly higher concentration of fibronectin

(225.77 ± 115.18 ng/mL) compared to Group II (8.04 ± 16.17 ng/mL).

Table VI: Comparison of fibronectin concentration between groups

Variable	Groups		p value
	Group I (n=60) Median (Range)	Group II (n=54) Median (Range)	
Fibronectin (ng/mL)	271.37 (45-502)	35.89 (0-65)	< 0.001

Data were analysed using Mann Whitney U test and level of significance was 0.05.

Diagnostic tests for PROM

Table VII summarizes the findings of different diagnostic tests for PROM. In nitrazine paper test, out of 60 patients in Group I, 49 (81.65%) were diagnosed of having PROM and 11 patients were negative for the test. In fern test, 53 (88.30%) patients out of 60 in Group I demonstrate positive results and 7 demonstrate negative results. In this study, positive results of both nitrazine test and fern test (double positive) were taken as the gold standard for confirmation of diagnosis of PROM. Out of the 60 patients, 47 were found double positive in the test and 13 were found double or single negative for nitrazine and fern tests.

Table VII: Comparison of diagnostic tests for PROM

Diagnostic Tests	PROM		Total
	Positive	Negative (Single/Double)	
Nitrazine paper test	49	11	60
Fern test	53	7	60
	47*	13**	60

*Double positive **Single or double negative

Fibronectin measurement as a diagnostic test for detecting PROM

Table VIII and IX show the value of fibronectin measurement as a diagnostic test for detecting PROM cases. The sensitivity of vaginal fibronectin, at cut off value of = 50 ng/mL, in correctly diagnosing PROM of those who have the disease is (45/47) × 100 = 95.7%, while the specificity of the test in correctly differentiating those who do not have the disease is (12/13) × 100 = 92.3%. The positive predictive value (PPV) of the test is (45/46) × 100 = 97.8% and the negative predictive value (NPV) of the test is (12/14) × 100 = 85.7%. The percentage of false +ves and false –ves yielded by the test are (1/46) × 100 = 2.2% and (2/14) × 100 = 14.3%. Finally the diagnostic accuracy of the test as computed from the data is (45 + 12)/(45 + 1 + 2 +12) ×100 = 57/60 × 100 = 95%.

Table VIII: Distribution of PROM subjects based on vaginal fibronectin level and combined fern and nitrazine paper tests

Vaginal fibronectin	Combined fern and nitrazine tests		Total
	Positive	Negative	
Positive (⩾ 50 ng/mL)	45	01	46
Negative (< 50 ng/mL)	02	12	14
Total	47*	13**	60

*Double positive **Single or double negative

Table IX: Diagnostic efficacy of fibronectin in detecting PROM

Components	Percentage
Sensitivity	95.7
Specificity	92.3
Positive predictive value	97.8
Negative predictive value	85.7
Accuracy	95

Discussion

Several methods have been used to diagnose the rupture of the membranes. These range from the traditional test of pooling, fern and nitrazine paper test to more invasive methods such as instillation of dye directly into the amniotic cavity. All above-mentioned procedures have higher percentage of false positive and false negative results or have hazards of invasive procedure. So it is essential to develop a test for diagnosis of PROM having high sensitivity, specificity and reasonably lower false positive and false negative results. With the focus of this idea, the present study was conducted to evaluate the diagnostic accuracy of fibronectin test.

In present study both Group I and Group II underwent fibronectin test. In Group I, mean concentration of fibronectin in vaginal fluid is 225 ± 115.18 ng/mL. On the other hand mean concentration of fibronectin in vaginal fluid is 8.04 ± 16.17 ng/mL in Group II subjects. So fibronectin concentration is very high in Group I and trace amount is present in Group II. So raised concentration of fibronectin in vaginal fluid indicates premature rupture of membrane.

Park et al[7] studied 78 women who received antenatal care and complained of fluid leakage prior to 37 weeks. Standard tests – vaginal speculum examination, nitrazine test, trans-abdominal sonography were performed. Rupture of membranes was diagnosed if any two of the standard tests were positive. Fibronectin in posterior vaginal fornix was determined qualitatively by ROM kit. They found in their series that sensitivity of the fibronectin test for prediction of ROM in the women who complained of fluid leakage was 90.6% and its specificity, positive and negative predictive value were 65.7%, 76.4% and 85.1% respectively. Twelve women did not have rupture of membrane on standard tests but were positive for fibronectin test.

Trovo et al[8] performed a study on PROM: Comparison of diagnostic tests. The objective of this study was to evaluate the accuracy of the vaginal pH test, the fern test, the fetal cells and of fetal fibronectin in vaginal discharge, which are used to diagnose PROM. They found that sensitivity, specificity and accuracy were respectively 70%, 97% and 90% for pH test; 70%, 100% and 93% for fern test; 50%, 93% and 82% for fetal cells; 100%, 90% and 93% for fibronectin test. Their sensitivity is a bit higher than the present study. They made conclusion that fibronectin test appeared to be the most sensitive and accurate. Salfelder et al[9] conducted a study on 133 pregnancies. Their observation had similar picture like present study regarding the value of fibronectin test to confirm the diagnosis of PROM.

Finally, the diagnostic accuracy of the test in our study was 95%. One patient not diagnosed as ruptured membranes based on standerd tests was positive in fibronectin test. It can be explained by prerupture stretching and consequent leaking of the membranes. The clinical significance of these 'false positives' requires further evaluation. Two patients diagnosed as ruptured membranes based on standard tests were negative for fibronectin test. This small number of false negative results may be due to flaws during sampling or technical difficulties in running the assay.

From the findings of this study it can be concluded that in cases of unequivocal rupture of the membranes, the result of the fibronectin test corresponds well with the clinical situation. So measurement of fibronectin in vaginal fluid is a sensitive test for detection of amniotic fluid in the vagina. However, further large scale studies are recommended to determine the usefulness of fetal fibronectin in the diagnosis of rupture of the membranes.

References

1. Gjoni M, Villar TJ. Preterm premature rupture of the membranes. 8th postgraduate course for training in reproductive medicine and reproductive biology. HRP-UNDP/UNFPA/WHO/WORLD BANK Special Programme. August, 2003.
2. Naef RW, Albert JR, Ross EL, Weber M, Martin RW, Morrison JC. Premature rupture of membrane at 34-37 weeks' gestation: aggressive versus conservative management. Am J Obstet Gynecol 1998; 178: 126-130.
3. Akter S. Maternal and fetal outcome in preterm premature rupture of membrane [FCPS Dissertation]. Dhaka: Bangladesh College of Physicians and Surgeons; 2006.
4. Medina TM, Hill DA. Preterm premature rupture of membranes: diagnosis and management. Am Fam Physician 2006; 73: 659-666.
5. Hellemans P, Verdonk P, Baekelandt M, Joostens M, Franex M, Gerries J. Preliminary results with the use of the ROM-check immunoassay in the early detection of rupture of the amniotic membranes. Euro J Obstet & Gynaeco Reprod Biol 1992; 43: 173-179.
6. Brubaker DB, Blict KE, Romlne M. Four immunoassay methods and standards compared for measuring fibronectin. Clin Chem 1987; 33(1): 126-129.
7. Park CW, Yoon TS, Lee JY, Park CJ, Kim YK, Jang BR et al. The usefulness of fetal fibronectin as a diagnostic method of preterm rupture of membrane. Korean J Perinatol 1997; 8(3): 291–294.
8. Trovo S, Brigato L, Plebani M, Brigato G, Grismondi GL. Premature membrane rupture. Comparison of diagnostic tests. Minerva Gynaecol 1998; 509(12): 519–522.
9. Salfelder A, Kagerah M, Nugent W, Hickel EJ. Detection of fibronectin for confirming the diagnosis of premature rupture of fetal membranes. Geburtshilfe Frauenheilkd 1992; 52(12): 730–733.