



Original Research Article

The Association between Bacterial Infections Including Bacterial Vaginosis and Premature Rupture of Membranes

M. Bharathi^{1*}, B. Pratibha², I. Jyothi Padmaja³

¹Assistant Professor, Dept. of Microbiology, Andhra Medical College, Visakhapatnam, AP, India.

²Senior Resident, Dept. of Obstetrics & Gynecology, Andhra Medical College

³Professor, Dept. of Microbiology, Andhra Medical College

*Correspondence Email: bharathikanthi65@gmail.com

Received: 22/09/2013

Revised: 26/10/2013

Accepted: 11/11/2013

ABSTRACT

Background: Premature rupture of membranes (PROM) occur approximately in 10% of all deliveries and responsible for maternal and fetal complications. Epidemiological data demonstrate an association between colonization of genital tract, by bacterial infections and increased risk of PROMs. PROMs tend to recur in subsequent pregnancies offers an opportunity for prevention.

Aim: To evaluate the association between bacterial infections including BV and PROM.

Materials and methods: Study group included 80 pregnant women with C/o leakage of liquor, with gestational age (GA) between 28-40wks. Control group included gestational age and parity matched 80 pregnant women without leakage of liquor. In both groups 40 women had GA of <37wks and 40 had GA between 37-40wks. After confirmation of rupture of membranes, high vaginal swabs were collected. After preparing smears, swabs were inoculated on mannitol salt agar, sheep Blood agar, MacConKey agar, chocolate agar and Sabouraud's dextrose agar. Pathogens were identified by standard methods. Smears were evaluated by Nugent's criteria for BV.

Results: Normal vaginal flora was seen only in 19 pregnant women (23.75%) in study group against 69 (86.25%) of control group. 15% of study group and 5% of control group were positive for BV. Pus cells were observed in 58.75% of PROM cases when compared to 10% of control group. Thirty nine of PROM cases (48.75%) were culture positive against seven of control group (8.75%). p value was significant in the above findings. Organisms isolated were *S. aureus* (20%), *Esch. coli* (13.75%), Group B Streptococci (8.75), Enterococci (2.5%) and *C. albicans* (3.75%). All babies born in PPROM cases were low birth weight babies.

Conclusions: Significant association was observed between PROM cases and bacterial infections. *S. aureus* was the most common organism isolated. BV was three times more common in PROM cases than in non-PROM deliveries. PPROM increased the risk of PTB and LBW.

Key words: Premature rupture of membranes, Bacterial vaginosis, Bacterial infections, Nugent's criteria

INTRODUCTION

Premature rupture of membranes (PROM) is characterised by rupture of membranes before onset of labor. PROMs occur approximately in 10% of all deliveries and result in the loss of normal protection of fetus and intrauterine contents from bacterial invasion. Consequently both the mother and fetus are at greater risk for infection.^[1] When membranes are ruptured prior to 37wks of gestation it is called preterm Premature rupture of membranes (PPROM) and it is associated with 30-40% of preterm deliveries and is an important cause of perinatal and neonatal morbidity and mortality.^[2,3]

Epidemiological data demonstrate an association between colonization of genital tract, by Group B Streptococci, Chlamydia trachomatis, Neisseria gonorrhoeae and microorganisms that cause bacterial vaginosis (BV) and increased risk of PPROMs.^[2] PPROMs tends to recur in subsequent pregnancies offers an opportunity for prevention.^[1] and antibiotic treatment significantly decreases the risk of PPROMs.^[2,4] Very few studies were available regarding association of bacterial infections other than BV and PROM. Hence we made an attempt to study the relation between bacterial infections including BV and PROM in our institute by that we can prevent PROM in the next pregnancy by screening and treating BV positive cases and other infections.

MATERIALS AND METHODS

Study group included 80 pregnant women with, complaint of leakage of liquor, gestational age between 28-40wks and cervical dilatation less than 3cm on examination from February 2011 to December 2011. Pregnant women with cervical encirclage, multiple gestation, polyhydramnios, antepartum hemorrhage, pregnancy induced hypertension, which are

known risk factors for PROM, were excluded. Control group included gestational age and parity matched 80 pregnant women without leakage of liquor. Institutional Ethics committee approval was taken.

Statistical analysis was done using Chi Square method.

After taking detailed obstetric history and H/o duration of leaking, physical examination was done to know gestational age, fetal position and presentation, number of fetuses and uterine action. Sterile speculum examination without antiseptic was done to confirm leakage of liquor. High vaginal swabs were collected after obtaining informed written consent. One swab was used to prepare smears and Gram's staining was done. Other swab was used for inoculation on mannitol salt agar, sheep Blood agar, MacConKey agar, chocolate agar and Sabouraud's dextrose agar to isolate Staphylococcus aureus, Streptococci, Enterobacteriaceae and non-fermentors, N.gonorrhoeae and Candida spp. After incubation at 37⁰C over night, plates were examined for growth. Pathogens were identified by colony morphology, Gram's reaction and biochemical characters as per standard methods.^[5]

Stained smears were examined under oil immersion objective of compound microscope for the presence of epithelial cells, pus cells, clue cells and microorganisms. Smears were evaluated by Nugent's criteria to diagnose BV. A grading system for Gram's stain of vaginal discharge has been developed by Nugent et al (1991) for the diagnosis of BV.

Score	Organism morphotype		
	Lactobacillus like	Gardnerella/ Bacteroides like	Mobiluncus like
0	>30	0	0
1	5-30	>1	1-4
2	1-4	1-4	>5
3	<1	5-30	-
4	0	>30	-

If the score was 7-10 after adding all scores, it was diagnosed as BV. Score of 0-3 suggests normal flora and in between equivocal. Amsel's criteria were not considered here because the alkalinity of vaginal secretions might be due to amniotic fluid leak, conduct of whiff test is subjective and 40% of BV cases are asymptomatic.

RESULTS

Thirty five pregnant women of both study and control group were primi and 45 were multi gravida. Fifty five pregnant women in study group and 60 in control group were booked cases. More than 50% of subjects in both groups belonged to lower economic class. Five of the study group had PROM in their previous pregnancy and two had PTB. Two of control group had PROM and one had PTB in their previous pregnancies. Findings of Gram's smear, culture and other characters were shown in table 1. Number of BV positive cases in relation to parity and gestation were shown in table 2 & 3.

Table 1. Showing microscopical, cultural and other characteristics of the subjects.

Characteristics	Study group (n-80) n (%)	Control group (n-80) n (%)
Registration status		
Booked	55 (68.75)	60 (75)
Unbooked	25 (31.25)	20 (25)
Economic status		
Lower class	50 (62.5)	45 (56.25)
Middle class	30 (37.5)	35 (43.75)
Upper class	0	0
In multi gravida		
H/o PROM	5 (11.11))	2 (4.44)
H/o PTB	2 (4.44)	1 (2.22)
Findings of Gram's smear		
Epithelial cells*	16 (20)	68 (85)
Pus cells*	47 (58.75)	8 (10)
Clue cells**	17 (21.25)	5 (6.25)
Gram positive bacilli***	19 (23.75)	69 (86.25)
Gram positive cocci	25 (31.25)	5 (6.25)
Gram negative cocci	0	0
Gram negative bacilli	11 (13.75)	3 (3.75)
Yeast cells	3 (3.75)	1 (1.25)
Culture +ve for pathogens*	39 (48.75)	7 (8.75)
S.aureus	16 (20)	3 (3.75)
Group B Streptococci	7 (8.75)	1 (1.25)
Escherichiae coli	11 (13.75)	2 (2.5)
Enterococci	2 (2.5)	0
Candida albicans	3 (3.75)	1 (1.25)
BV positive cases****	12 (15)	4 (5)

p value <0.0005 ** p value <0.01 *** p value <0.0005 **** p value <0.05

Table 2. Showing BV positive cases in both groups.

Group	Category	No. tested	BV +ve	Total BV +ve cases
Study group with PROM	Primi	35	5 (14.28%)	12 (15%)
	Multi	45	7 (15.55%)	
Control group Without PROM	primi	35	1 (2.85%)	4 (5%)
	multi	45	3 (6.66%)	

p value significant between two groups (< 0.05)

Table 3. Showing BV positive cases in PPROM & PROM cases.

Gestational age in wks	No. tested	BV +ve cases
< 37wks (PPROM)	40	8 (20%)
37-40wks (PROM)	40	4 (10%)

p value not significant.

DISCUSSION

Infections of genital tract are one of the possible causes for PROM. One possible mechanism is ascending infection results in replication of bacteria in placenta, decidua and membranes. [3] Another is, Group B Streptococci, *Staphylococcus aureus*, *Trichomonas vaginalis* and microorganisms that cause bacterial vaginosis secrete proteases that degrade collagen and weaken the fetal membranes, leading to PROM. [2,3] Recent studies have emphasized the importance of BV as a risk factor for PROM. BV is an imbalance of normal vaginal flora with an over growth of anaerobic bacteria and lack of the normal lactobacillary flora [4] and is associated with a 2.6 fold risk for preterm labor (PTL), 6.9 fold risk for preterm birth (PTB) and a 7.3 fold risk for preterm Premature rupture of membranes. [6]

PROMs occur more frequently among poor or single women, women who smoke and teenagers. Such women are also at greater risk for STDs and a higher incidence of colonization with Group B Streptococci and bacterial vaginosis. [1] 62.5% of subjects(50) in study group belonged to lower economic class. None of the pregnant women were smokers and only 15% were teenagers. Recurrence risk of PPROM is 16-32% as compared with 4% in women with a prior uncomplicated term delivery. [7] H/o PROM was present in 5

(11.11%) cases in this study. Host inflammatory response to bacterial infection is mediated by polymorphs and macrophages, that induce cytokines, prostaglandins result in PROM.^[2] In the study group, pus cells were present in 58.75% of Gram's stained smears, suggested the presence of bacterial infection. But culture was positive only in 48.75% cases. We could not isolate organisms in 10% of cases. This might be due to anaerobic infection or infections of Chlamydia / Mycoplasma / Ureaplasma, which are non cultivable by ordinary methods.

Normal vaginal flora was seen in 19 cases (23.75%) of study group and 69 cases (86.25%) of controls. In study group clue cells were observed in 17 cases (21.25%), but BV was diagnosed only in 12 cases (15%) according to Nugent's criteria. Gram's staining findings correlated with culture findings. Gram positive cocci were more common (31.25%) than Gram negative bacilli (13.75%), with *S.aureus* in 16 (20%) cases. Strikingly Gram negative cocci were absent. One reason for this might be, pregnant women with gonorrhea seeks medical advice promptly, because of symptoms. We isolated *S.aureus* (20%), Group B Streptococci (8.75%), *Esch.coli* (13.75%), Enterococci (2.5%) and *Candida albicans* (3.75%) in study group without much difference between PROM and PPRM cases. We isolated the same organisms, except Enterococcus, in the control group, in small number of cases. We did not isolate any non-fermentors. Karat et al also isolated Enterobacter and Proteus mirabilis in addition to the above organisms and in more number of cases except for Group B Streptococci.^[3] Micamo et al isolated Staphylococcus haemolyticus, Streptococcus agalactiae, Esch.coli, Fusobacterium nucleatum and Prevotella bivia.^[8]

BV is significantly associated with increased risk of PTB and PROM.^[9,10] In the present study 20% of PPRM cases and 10% of PROM cases were positive for BV, where as it was 16% in a study by C.Karat et al (in >31wks gestation) and 21.53% in a study by Indulatha et al (PROM) and at higher rates than the present study.^[3,11] In PPRM it was 26.82% in Azam Azargoon study,^[12] almost comparable to our study, but a very higher rates in Micamo et al study (72.9%) and in a study by Sima Gity (64.29%).^[8,13] Where as Ziaei S et al found no significant association between BV and PROM.^[14] Wide variation in the results might be due to differences in diagnosis methods and differences in study population. High percentage of BV in PPRM cases than PROM cases strongly suggest the adverse effects of BV in pregnancy. Induction of labor in PPRM is recommended when the gestational age is more than 34wks. With malpresentations due to cord prolapse, the risk of caesarian delivery with its surgical risks to the parturient, is higher in PPRM as compared with term deliveries.^[7] Labor was induced in eight PPRM cases. Caesarian section was done in three PPRM cases and in six PROM cases. Sita Rama Shrestha & Paban Sharma in their study observed 70% spontaneous, 3.5% instrumental and 27% caesarian deliveries,^[15] where as it was 78.75%, 10% and 11.25% respectively in this study.

PPRM is a major cause of perinatal morbidity and mortality. It is associated with 20- 30% of all preterm births (PTB) and prognosis is related to gestational age at presentation and delivery.^[7] All babies born in PPRM cases were low birth weight babies (19 babies with <2kgs and 21 between 2 & 2.5kgs) and had risks of LBW. In PROM cases 29 babies had a weight of 2.5-3kgs and 11 had >3kgs).

High percentage of bacterial infections including bacterial vaginosis, in PPRM and PROM cases when compared to non-PROM pregnancies, suggested their role in the etiology of premature rupture of membranes and indirectly responsible for the risks faced by babies due to PTBs and LBW and in mothers for risks of infection and surgery. But postpartum infections were not present in the present study as in the study of Borna S,^[16] may be due to antibiotic cover. Antibiotic prophylaxis was given to pregnant women after diagnosis of PROM (Ampicillin 500mg BD). After getting culture & sensitivity report, treatment was continued or changed based on sensitivity reports. But screening of asymptomatic pregnant women for bacterial infections and BV is in debate. Because it may induce iatrogenic infection and lead to financial burden on individuals and government. Some advocated screening and treatment,^[3,9] and some were against it.^[17] still more studies are necessary to come to a common conclusion to benefit both mother and neonate.

Limitations: Less sample size; needs further study involving more number of PROM cases. Wet mounts were not examined for *T. vaginalis*, due to delay in transporting sample to the laboratory. Pathogens were isolated only by culture, but not by antigen detection and molecular methods due to financial constraints. Screening for bacterial infections was not done prior to rupture of membranes.

CONCLUSIONS

Significant association was observed between PROM cases and bacterial infections. *S. aureus* was the most common organism isolated. BV was three times more common in PROM cases than in non-PROM pregnancies. PPRM increased the risk of PTB and LBW.

AKNOWLEDGEMENTS

We are thankful to Dr. Krishnaveni, Professor, Dept. of Community Medicine for her guidance in statistical analysis.

REFERENCES

1. Pedro A Poma “Premature rupture of membranes” Journal of National Medical Association, 1996; 88 (1): 27-32.
2. Parry S, Strauss JF “Premature rupture of the fetal membranes” The New England Journal of Medicine, 1998; 338 (10): 663-70.
3. C. Karat, P. Madhivanan, K. Krupp, S. Poornima, NV Jayanthi, JS Suguna, E. Mathai “ The Clinical and Microbiological correlates of Premature rupture of membranes” IJMM, 2006; 24 (4): 283-5.
4. McDonald H, Brocklehurst P, Parsons J, Vigneswaran R “ Antibiotics for Treating Bacterial Vaginosis in Pregnancy” Cochrane Database Syst. Rev. 2005: (1) CD000262
5. Mackie & McCartney (2008) Practical Medical Microbiology. 14thed, 2008: Publisher Churchill Livingstone. Chapters 7,11,12,20 :pp 131-149, 253,267,269-271,361.
6. Tapio Kurki, Aulikki Sivonen, Olli-Veikko Renconen, EEva Savia and Olavi Ylikorkala “Bacterial Vaginosis in early pregnancy and pregnancy outcome” Obstetrics & Gynaecology, August 1992; 80 (2): 173-7.
7. Aaron B Caughey, Julian N Robinson, Errol R Norwitz “ Contemporary diagnosis and management of Preterm Premature rupture of membranes” Reviews in Obstetrics & Gynecology, 2008; winter 1 (1):11-12.

8. H. Micamo, Y. Sato, Y. Hayasaki, K. Kawazoe, Y X Hua, T Tamaya “Bacterial isolates from patients with preterm labor with and without preterm rupture of the fetal membranes” *Infectious Diseases in Obstetrics & Gynaecology*, 1997; 7 (4): 190-4.
9. Mc Gregor JA, French JI, Parker P, Draper D, Patterson E, Jones W, Thorsgard K, Mc Fee J “Prevention of Premature birth by Screening and Treatment for Common Genital tract infections: Results of a prospective controlled evaluation” *Am J Obstet Gynecol* 1995; 173 (1): 157-67.
10. Purwar M, Ughade S, Bhagat B, Agarwal V, & Kulkarni H “Bacterial vaginosis in early pregnancy and adverse pregnancy outcome” *Journal of Obstetrics and Gynaecology Research*, 2001; 27 (4):175-181.
11. Indu Latha, Yashodhara Pradeep, Sujatha and Amita Jain “ Estimation of the Incidence of Bacterial Vaginosis and other vaginal Infections and its consequences on maternal / fetal outcome in pregnant women attending an antenatal clinic in a tertiary care hospital in North India” *Indian Journal of Community Medicine*, 2010, 35 (2): pp 285-9.
12. Azam Azargoon, Shohreh Darvishzadeh “Association of Bacterial Vaginosis, *Trichomonas vaginalis* and Vaginal acidity with outcome of pregnancy” *Arch Iranian Med* , 2006; 9 (3): 213-7.
13. Sima Gity “ Prevalence of the Bacterial Vaginosis and Group B Streptococcus in Term and Preterm Pregnancies” *OBGYN.net*, Oct 26, 2011; available at S Gity-hcp.obgyn.net
14. Ziaei S, Sadrkhanlu M, Moeini A, Faghihzadeh S “Effect of Bacterial Vaginosis on premature rupture of membranes and related complications in pregnant women with a gestational age of 37-42 weeks” *Gynecol Obstet Invest*, 2006;61(3):135-6.
15. Sita Rama Shrestha & Paban Sharma “Fetal outcome of pre-labor rupture of membranes” *N J Obstet. Gynecol* , 2006; 1 (2): 19-24.
16. Sedigheh Borna, Hajieh Borna, Sogra Khajardoost, Sedigheh Hantoush Zadeh “Perinatal outcome in Preterm premature rupture of membranes with Amniotic fluid index <5 (AFI<5)” *BMC Pregnancy and Child birth*, 2004;4:15 available at doi:10.1186/1471-2393-4-15
17. J Christopher Carey, Mark A Klebanoff, John C Hauth, Sharon L Hillier, Elizabeth A Thom et al “Metronidazole to prevent Preterm delivery in Pregnant women with Asymptomatic Bacterial Vaginosis” *N Engl J Med* 2000; 342: pp 534-540.

How to cite this article: Bharathi M, Pratibha B, Padmaja IJ. The association between bacterial infections including bacterial vaginosis and premature rupture of membranes. *Int J Health Sci Res.* 2013;3(12):58-63.
