

Original Research Article

Intravaginal Pessary Containing Lactobacillus, Clindamycin, Clotrimazole Decreases the Rate of Preterm Delivery and Incidence of Low Birth Weight in Asymptomatic Mothers with Abnormal Vaginal Flora in Early Pregnancy

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ABSTRACT

Preterm labour and low birth weight is a big concern in developing country like India. Abnormal vaginal flora in asymptomatic mothers has been found to be a causative factor in recent emerging evidences. The outcome gathered from various studies conducted in recent years have been remarkably consistent and have supported the hypothesis that antibiotic treatment before 20 weeks gestation reduces the risk of preterm birth. ^(1,2) Screening for abnormal vaginal flora can be safely carried out in pregnant women who have a previous history of preterm labour. This study was conducted in asymptomatic pregnant woman with such past history with intervention as intravaginal antibiotic and probiotic pessary and looked for maternal and fetal outcomes. The most common vaginal infection detected in this group was BV (23.4%). Candidiasis was present in 6.6% of pregnant women. A small number of pregnant women (0.4%) had both BV and Candidiasis. Treatment of asymptomatic women having abnormal vaginal flora in early pregnancy was associated with a statistically significant decrease in the rate of preterm delivery and low birth weight. Lactobacillus in addition causes further advantage in beneficial effect of intravaginal antibiotic pessary.

Key words: Intravaginal pessary, Preterm Labour, lactobacillus.

INTRODUCTION

Screening and treatment of abnormal vaginal flora during pregnancy to prevent preterm labour is a matter of debate for practicing obstetrician and physician in a country where maternal health and its outcome is a big concern. The recent evidence suggests that infection may be implicated in a substantial proportion of cases of preterm delivery. Neonatal morbidity and mortality is primarily influenced by gestational age and less so by birth weight. The microbial flora normally present in the human vagina plays a key role

in preventing pathological organisms, including those responsible for sexually transmitted diseases, yeast infections, bacterial vaginosis and urinary tract infection. The outcome gathered from various studies conducted in recent years have been remarkably consistent and have supported the hypothesis that antibiotic treatment before 20 weeks gestation reduces the risk of preterm birth. ^(1,2) It is concluded that screening for abnormal vaginal flora cannot be generalized but it may be safely carried out in pregnant women who have a previous history of preterm labour.

Antibiotics with lactobacillus treatment can eradicate abnormal vaginal flora in pregnancy however screening and treating all pregnant women with asymptomatic bacterial vaginosis to prevent preterm birth and its consequences is not substantiated by evidence. Furthermore, this was also hypothesised that is there any additional benefit by addition of lactobacillus to intravaginal pessaries.

Aims & objectives

The study was undertaken in early pregnant asymptomatic females having abnormal vaginal flora to correlate the presence of abnormal vaginal flora in asymptomatic pregnant women with maternal outcome like preterm birth, pre-labour rupture of membranes and infection related morbidity and neonatal morbidity and to study the association between use of antimicrobial vaginal suppository in asymptomatic pregnant women with abnormal vaginal flora and these maternal and neonatal outcomes.

MATERIALS AND METHODS

Study population: This study was conducted in Department of Obstetrics and Gynaecology and Department of Microbiology, Rajendra Institute of Medical Sciences, Ranchi, India. 500 asymptomatic pregnant women who presented to antenatal OPD, irrespective of their parity, were chosen for the study. All subjects gave informed consent and the study was approved by the board of studies.

Inclusion Criteria: Asymptomatic Pregnant women with gestational age between 13-24 weeks and with no obvious risk factors for preterm delivery. Period of gestation (POG) was calculated by correlating the clinically determined period of gestation with the date of last menstrual period (LMP) and/or ultrasound scan.

Exclusion criteria: Pregnant women symptomatic for vaginal infection or with associated complications / risk factors for preterm delivery (twin gestation, bleeding per vaginum in first trimester, essential hypertension, congenital uterine anomalies)

or with known medical disorders like diabetes mellitus, heart disease, renal disease, autoimmune disorders were excluded from the study.

Study design: This study was a prospective randomized controlled trial. 500 asymptomatic pregnant women were recruited in the study and were followed up to pregnancy outcome (flow chart1).

RESULTS

Age distribution: The study group comprised of 500 pregnant females with mean age study group was 23.84±3.27 yrs.

The minimum age was 18 yrs and maximum age was 36 yrs. The maximum number of women fell between age group 20-24 yrs (49.6%). Mean age of women in intervention group was 24.01±3.24 yrs and in control group was 23.68±3.32 yrs.

Table 1: Age distribution

Age (Years)	No.(n=500)	Percentage (%)
<20	37	7.4
20-24	248	49.6
25-29	143	29.6
30-34	60	12
>35	7	1.4

Population distribution based on gravida (Table 2)

The distribution of total study group based on gravida is depicted in table 2. Maximum number of women in study was primi gravidas (49.2%). The gravida distribution in intervention and control group also followed the same pattern.

Table 2: Gravida distribution of study n=500

Gravida	No. (n=500)	Percentage (%)
G1	246	49.2
G2	142	28.4
G3	77	15.4
>G4	35	7

Period of gestation

The mean period of gestation (POG) by abdominal examination was 17.89±3.01 weeks which corresponded to the period of gestation calculated by the date of last menstrual period. Mean POG by the date of last menstrual period and/or was 17.64±2.39 weeks. The minimum POG was 13 weeks and the maximum was 23 weeks.

Local examination

Routine inspection of the vulva followed by a per speculum examination of the vagina and cervix was performed for all the pregnant women. The findings of local examination are depicted in table 3.

Vulva

The vulva was inspected to detect any abnormality evidence of vulvitis, erythema was interpreted as unhealthy. The distribution of pregnant women based on these findings is depicted in table 3.

Table 3: Vulva

Condition	No. (n=500)	Percentage
Healthy	478	95.6
Unhealthy	22	4.4

Despite the fact that only asymptomatic women were included in the study, the vulva was found to be unhealthy in 22 women (4.4%)

Vagina

On per speculum examination, vaginal walls were healthy and vaginal discharge was present in all women. The physical characteristics of vaginal discharge on per speculum examination were different and are summarized in table 4.

Table 4: Characteristics of vaginal discharge

	No. (n=500)	Percentage (%)
Quantity		
• Scanty	369	73.8
• Moderate	131	26.2
Consistency		
• Mucoid	300	60
• Thin homogenous	144	28.8
• Thick	179	22.4
Odour		
• Odorless	474	94.8
• Present	26	5.2

On per speculum examination, vaginal discharge was found to be scanty in amount in 73.8% and moderate in 26.2%. Mucoid discharge suggestive of physiological response to pregnancy was present in 60%. Thin homogenous vaginal discharge characteristic of bacterial vaginosis (BV) was present in 28.8% and thick discharge suggesting candidiasis was present in 22.4%. Vaginal discharge was odourless in 94.8% and had a fishy odour in 5.2%.

Table 5: Gram stain findings of Vaginal smear

	No. (n=500)	Percentage (%)
Clue cells		
• Present	98	19.6
• Absent	402	80.4
Yeast		
• Present	35	7
• Absent	465	93
PMNs (>10/HPF)		
• Present	85	17
• Absent	415	83

Gram stain findings of vaginal smear

The vaginal smears of all the 500 pregnant women were Gram stained. The smear was evaluated for the presence of abnormalities such as clue cells, yeast, polymorph nuclear cells (PMNs) and the presence of BV. The findings of Gram stain are depicted in table 5 and 6.

On microscopic examination of the Gram stained vaginal smear, clue cells indicative of BV was present in 19.6% of the pregnant women. Budding yeast cells or pseudo hyphae indicative of candidiasis were present in 7% polymorph nuclear leucocytes (PMNs) >10/HPF indicative of either trichomonas vaginitis or infection from the cervix was present in 17% of the pregnant women.

Diagnosis of Bacterial Vaginosis (BV)

The diagnosis of BV was made using Gram stain scoring system proposed by Nugent et al, (3) According to this; the vaginal smears were examined for the bacterial morphotypes and scored. The various bacterial morphotypes identified were as shown in table 15 and the population distribution on the basis of this categorization is depicted in table 6.

Table 6: The categories of vaginal micro flora as evaluated by vaginal smear Gram stain (Nugent et al)

	No. (n=500)	Percentage (%)
Non BV (0-3)	331	66.2
Intermediate (4-6)	52	10.4
Bacterial vaginosis (≥ 7)	117	23.4

On evaluation of the Gram stained vaginal smear by Nugent's criteria, BV was found to be present in 23.4% of the pregnant women. Intermediate BV was found to be present in 10.4% and no evidence of BV was detected in 66.2% of pregnant women.

Incidence of abnormal vaginal flora

The overall incidence of vaginal infections was calculated on the basis of Gram stain findings of vaginal smear. The distribution of population on the basis of presence of vaginal infections is depicted in table 7

Table 7: Incidence of abnormal vaginal flora

	No. (n=500)	Percentage (%)
Bacterial vaginosis	117	23.4
BV + Candidiasis	2	0.4
Candidiasis	33	6.6
Total	152	30.4

In accordance with the findings on Gram stain of the vaginal smear, BV was detected in 117 (23.4%) of the pregnant women. Candidiasis was present 33 (6.6%) of the pregnant women. 2 pregnant women (0.4%) had evidence of presence of both Candida and BV. The overall incidence of abnormal vaginal flora in this study population was found to be 152 (30.4%) whereas as many as 69.7% of the total population had no evidence of vaginal infection.

Distribution of pregnant women with vaginal infections into intervention and control groups

The total pregnant women diagnosed with vaginal infection were randomized into two groups viz. intervention and control groups. The distribution is depicted in table 8.

Table 8: Distribution of pregnant women with vaginal infection on the basis of treatment given.

Treatment	No. of patient	Percentage (%)
Total no of patient with abnormal vaginal flora	152	30.4
Intervention group	76	50
Control group	76	50

152 pregnant women detected to have vaginal infection, were randomized into an intervention and a control group using a computer programme. Accordingly, 76 pregnant women with vaginal infection received treatment (intravaginal pessary containing lactobacillus, clindamycin, clotrimazole for 6 days) and the remaining 76 pregnant women did not undergo any intervention.

Repeat Smear: Repeat vaginal smear were taken after 3 weeks and Gram Staining done from the pregnant women in intervention group to look for eradication or persistence of abnormal vaginal flora.

The results have been shown in Table 9.

Table 9: Persistence of abnormal vaginal flora in repeat smear - (by Nugent et al)

Repeat smear	No. (n=76)	Percentage (%)
Non BV (0-3)	55	72.3 %
Intermediate (4-6)	9	11.8 %
Bacterial vaginosis ≥ 7	12	15.7 %

On evaluation of repeat vaginal smear by gram staining in accordance with nugents criteria, persistence of BV was seen in 15.7% of the pregnant women. It was completely cured in 72.3% of cases. Repeat smear was taken and gram staining done 3 weeks after the course of oral metronidazole and ampicillin. The result has been depicted in table 10.

Table 10: Persistence of abnormal vaginal flora after the course of oral metronidazole and ampicillin

Repeat smear	No. (n=12)	Percentage (%)
Non BV (0-3)	7	58.3 %
Intermediate (4-6)	2	16.6 %
Bacterial vaginosis (≥ 7)	3	25 %

After the course of oral metronidazole and ampicillin, 58.3% of the pregnant women with persistence of abnormal vaginal flora had complete cure. In 25% of the women the abnormal vaginal flora persisted in spite of oral metronidazole and ampicillin. All the patients in this group had preterm delivery.

Outcome parameters in intervention and control group

Patient with abnormal vaginal flora were randomized into an intervention group and a control group. The details of pregnancy outcomes in these two groups are summarized below.

Maternal

The maternal outcome measures in the intervention group (pregnant women with abnormal vaginal flora who received medication) and the control group (Pregnant women with abnormal vaginal flora with no intervention) in terms of period of gestation at delivery, evidence of PROM are depicted in tables 11, 12 and 13.

Table 11: Comparison of incidence of preterm delivery (POG <37) in intervention and control group

	Intervention (n=76)		Control (n=76)		'p' value
	No.	%	No.	%	
Preterm delivery	9	11.8	20	26.3	0.0390

The rate of preterm delivery was found to be 11.8% in intervention group & compared to 26.3% in control group. The difference was 14.51% (95% CI: 2.24-26.78) P value is 0.0390 i.e. <0.05 so is significant. Suggesting that treatment of abnormal flora leads to a significant reduction in rate of preterm delivery. The distribution of preterm delivery according to gestational age is depicted in table 12.

Table 12: Comparison of preterm birth (28 to 37weeks) in pregnant women abnormal vaginal flora

Gestational age (weeks)	Intervention (n=76)		Control (n=76)		P value
	No.	%	No.	%	
28-30 wk	Nil	-	1	1.3	
31-33 wk	1	1.3	8	10.5	0.0392
34-36 wk	8	10.5	12	15.7	0.4716

Preterm delivery was seen mostly at 34-36wk of gestational being 10.5% in intervention & 15.7% in control group. The incidence of preterm deliveries was decreased in the intervention group. The decrease in the incidence of preterm delivery at period of gestation between 31 and 33 (1.3% Vs 10.5%) was statistically significant (p<0.05).

Flow charts showing follow up of pregnant women in the study (Flow chart 1)

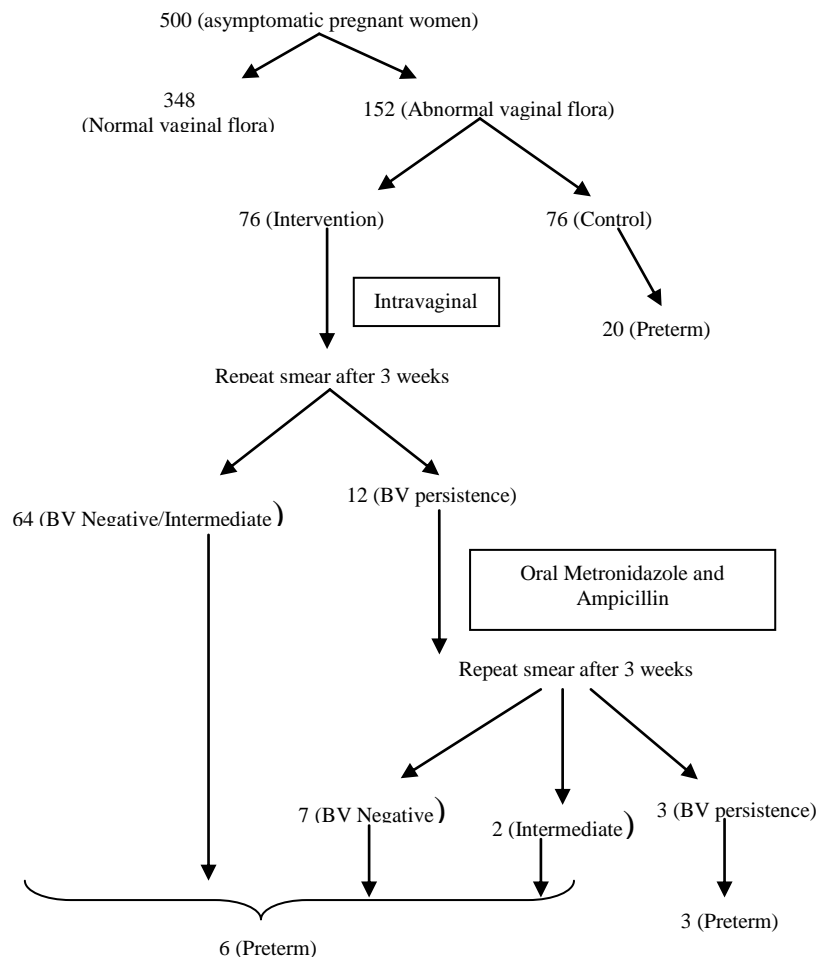


Table 13: Adverse maternal outcomes in both groups of pregnant women with abnormal vaginal flora

	Maternal outcome	Intervention (n=76)		Control p (n=76)		P value
		No.	%	No.	%	
1	Late miscarriage (20-28 weeks)	2	2.6	5	6.5	0.4390
2	Preterm delivery (28-37 weeks)	9	11.8	20	26.3	0.0390
3	PROM	6	7.8	10	13.1	0.4278
4	Puerperal pyrexia	7	9.2	09	11.8	0.7916

Table 14: Adverse fetal outcome in both groups of pregnant women with abnormal vaginal flora

	Fetal outcome	Intervention (n=74)		Control (n=71)		P value
		No.	%	No.	%	
1	Low birth weight (<2.5 kg)	10	13.5	20	28.1	0.0485
2	Birth asphyxia	3	4	5	7	0.6715
3	NICU admission	8	10.8	13	18.3	0.2953

Preterm delivery was seen in 11.8% of intervention group & 26.37% of control group. This correlation between rate of preterm delivery in intervention and control group was statistically significant with P=0.0390. However the decrease in rate of late miscarriage, PROM, puerperal pyrexia in intervention group was not statistically significant.

Correlation of adverse fetal outcome in both groups of pregnant women with abnormal vaginal flora (intervention and control group)

The rates of low birth weight, birth asphyxia and admission to neonatal ICU (NICU) among neonates born to women who continued pregnancy beyond 28 weeks were compared in the two groups. These

were a total of 74 pregnant women in the intervention group and 71 pregnant women in the control group. Their details are depicted in table 14.

The decrease in the incidence of low birth weight in the intervention group was found to be statistically significant (P<0.05). However, the decrease in the rate of birth asphyxia and NICU admission was not found to be statistically significant (P>0.05).

Overall, abnormal vaginal flora was associated with greater maternal and fetal morbidity. Treatment of this problem conferred a statically significant benefit. The diagnosis of BV was made using Gram stain scoring system proposed by Nugent (table 15)

Table 15: Nugent's criteria

I	II	III
Lactobacillus morphotypes - large gram +ve rods/HPF Score :- 0→>30 1→ 15-30 2→ 1-14 3→<1 4→ 0	Gardnerella - small gram -ve variable rods Bacteroides - small gram variable rods Score :- 0→ 0 1→<1 2→ 1-4 3→ 5-30 4→>30	Mobiluncus - Curved gram variable rods Score :- 0→ 0 1→<5 2→ 5+

Total score - I + II + III, 0-3 - Negative for bacterial vaginosis, 4-6 - Intermediate, 7+ - Bacterial vaginosis. The population distribution on the basis of this categorization is depicted in table 6.

DISCUSSION

Infection and spontaneous preterm labour is closely associated and it is responsible for preterm birth in up to 40% of cases. (2) A more logical step would be to identify a group of women at risk and to intervene using antibiotic prophylaxis. The justification for this study is that several trails have suggested a beneficial effect of antibiotic use in reducing the rate of preterm birth in patient at risk when administered early during pregnancy. The prevalence of

younger age group in this study is likely to be a reflection of early age of marriage and first conception in Indian women.

The evidence of abnormalities detected on the examination findings even in asymptomatic low risk women could be contributed to the ignorance of these pregnant women and their perception of these abnormalities as normal changes in pregnancy. The most common vaginal infection detected in this group was BV (23.4%). Candidiasis was present in 6.6% of pregnant women. A small number of pregnant women (0.4%) had both BV and Candidiasis.

The pregnant women in intervention group received intravaginal Lactobacillus, clindamycin and clotrimazole as a

combination pessary. This was in contrast to other studies. ⁽⁴⁻⁶⁾ which used only vaginal clindamycin cream in 2% concentration as the treatment. In contrast, Ugwumadu et al ⁽⁷⁾ administered oral clindamycin to the intervention group. In two trials, ^(8,9) metronidazole was the choice of drug used for the intervention. The new component of the vaginal pessary that was used in this study is probiotics i.e. Lactobacillus sp. Lactobacilli are the dominant bacteria of vaginal flora and possess antimicrobial properties that regulate other urogenital microbiota. Use of lactobacillus containing probiotic to restore commensal vaginal flora has been proposed for treatment and prophylaxis of bacterial urogenital infection. The actual mechanism of action of probiotics in vagina is multifactorial. The production of lactic acid, bacteriocins and hydrogen peroxide seems to be important and modulation of immunity is another possible mechanism. These probiotics have been shown to decrease the risk of bacterial vaginosis. In two studies, ^(10,11) intravaginal lactobacillus suppositories had increased cure rates for BV when compared to placebo. So a combination pessary was used to see for the decrease in preterm delivery rates along with the added advantage of Lactobacillus. The results in this study were in contrast to earlier studies ^(9,12,13) which did not report a significant decrease in the rate of preterm delivery after the use of antibiotics. In this study, treatment was started within two weeks after randomization i.e. by 19.6 weeks, hence leading to a significant decrease in the rate of preterm delivery.

CONCLUSION

The overall incidence of abnormal vaginal flora in asymptomatic pregnant women was 30.4%. Bacterial vaginosis was the most common (23.4%) vaginal infection encountered in pregnant women. Treatment of abnormal vaginal flora in asymptomatic pregnant women by intravaginal pessary containing lactobacillus, clindamycin, clotrimazole caused complete cure in 72.3%

and there was persistence of bacterial vaginosis in only 15.7%. Treatment of asymptomatic women having abnormal vaginal flora in early pregnancy was associated with a statistically significant decrease in the rate of preterm delivery but not with decrease in late second trimester miscarriage, PROM and puerperal pyrexia. Preterm birth between 31-34 weeks of gestation was significantly lowered after treatment of abnormal vaginal flora in early pregnancy. Treatment of asymptomatic mothers with abnormal vaginal flora in early pregnancy was also associated with a statistically significant decrease in the incidence of low birth weight in their babies. Addition of lactobacillus to the intravaginal pessary gives an added advantage to treat bacterial vaginosis and decrease preterm delivery rates when compared to previous studies. It is therefore appropriate to conclude that integrating a simple infection screening programme for abnormal vaginal flora in asymptomatic pregnant women into routine antenatal care is a low cost primary preventive measure that can be aimed at decreasing the incidence of preterm labour. The treatment of these women in early second trimester is an effective method of secondary prevention of preterm births and low birth weight infants.

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