Pregnancy outcome in asymptomatic women with abnormal vaginal flora without any treatment and after treatment with vaginal clindamycin and clotrimazole: A randomised controlled trial

S Gupta,[1] MB BS, MD; **R Tripathi**,[1] MB BS, MD; **N Singh**,[1] MB BS, MS; **P Bhalla**,[2] MB BS, MD; **S Ramji**,[3] MB BS, MD; **Y M Mala**,[1] MB BS, MD

[1] *Department of Obstetrics and Gynaecology, Maulana Azad Medical College, New Delhi, India*
[2] *Department of Microbiology, Maulana Azad Medical College, New Delhi, India*
[3] *Department of Paediatrics, Maulana Azad Medical College, New Delhi, India*

Corresponding author: *N Singh (nilanchalisingh@gmail.com)*

Aims. To study the role of screening for and treatment of abnormal vaginal flora in early pregnancy, and its correlation with pregnancy outcome.

Methods. Eight hundred asymptomatic women seen at the antenatal clinic of Lok Nayak Hospital, New Delhi, India, at 12 - 24 weeks' gestation were screened for abnormal vaginal flora by means of examination of vaginal fluid smears on Gram-stained slides. Two hundred and forty-two women with abnormal vaginal flora were allocated randomly to receive either treatment (vaginal clindamycin and clotrimazole) or no treatment. The presence of abnormal vaginal flora was correlated with pregnancy outcomes in terms of preterm delivery or late miscarriage, premature rupture of the membranes (PROM) and puerperal sepsis.

Results. A total of 242 patients with abnormal vaginal flora for whom outcome data were complete were analysed. Intervention in women with abnormal vaginal flora was associated with a decrease in the rate of preterm delivery (30.3% v. 18.6%; relative risk 1.65; 95% confidence interval 1.04 - 2.63; $p<0.05$). The advantage did not extend to late miscarriage, PROM or puerperal sepsis, as the decrease in these outcomes did not attain statistical significance.

Conclusions. Screening for and treatment of asymptomatic abnormal vaginal flora in early pregnancy significantly reduces the rate of preterm delivery and consequent perinatal morbidity and mortality.

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Preterm delivery (birth before 37 completed weeks of gestation) is one of the leading causes of neonatal morbidity and mortality. Despite improved neonatal care, 70 - 80% of all perinatal deaths among neonates without congenital malformations occur in infants born prematurely. However, the causes of preterm labour are poorly understood. There is evidence that maternal genital tract infections and subclinical amniotic infections may be responsible for preterm delivery in up to 40% of cases.[1,2]

The most common pathway of intrauterine infection and resultant amniotic infection is thought to be from the cervix and vagina. This ascending infection into the uterine cavity from the lower genital tract can result in deciduitis, chorioamnionitis, amniotic fluid infection, fetal sepsis and intrauterine death. Intrauterine infection may occur early in pregnancy and remain asymptomatic and undetected for months until preterm labour or premature rupture of the membranes (PROM) occur.[3]

Abnormal genital tract colonisation detected early in pregnancy has been found to revert to normal, but is still associated with an increased risk of adverse pregnancy outcome.[4]

Maternal genital tract colonisation or infection may be one of the most important modifiable risk factors for early delivery. It may therefore be possible to prevent a proportion of preterm births by screening pregnant women for abnormal vaginal flora and eradicating it in early pregnancy before inflammation and tissue damage occur.

Material and methods

This study was a prospective, randomised controlled trial and was approved by our institutional review board. After obtaining ethical clearance, 800 asymptomatic women seen at the antenatal clinic of Lok Nayak Hospital, New Delhi, India, at 12 - 24 weeks' gestation were included in the study. With a 4% margin of error at 95% confidence interval (CI), the estimated number of patients required for this study was 600. The gestational age range chosen was wide, as very early booking is not common in our antenatal clinic and if only patients in early pregnancy were recruited, far fewer would have been available to enrol in the study. The patients were enrolled consecutively within one unit and included in the study if they fulfilled the selection criteria. Patients with symptomatic vaginal infections, obvious risk factors for preterm delivery such as twin gestation, vaginal bleeding

in the first trimester, essential hypertension, autoimmune disorders, congenital uterine abnormalities, antiphospholipid syndrome, diabetes mellitus or a history of cone biopsy of the cervix were excluded.

Gestational age was calculated from the first-trimester ultrasound scan if the patient had had one. If no scan was available but the patient was sure of her last menstrual period (LMP) and had previously had regular cycles, we used the LMP to calculate gestational age.

All patients gave written informed consent, and then underwent a detailed antenatal checkup. In addition, they were screened for the presence of abnormal vaginal flora. The external genitalia were examined for any signs of inflammation or vulvar erythema. The vagina was examined with a speculum and the condition of the vaginal wall and the nature of the vaginal fluid (amount, colour, consistency, odour) were noted. Using a sterile cotton-tipped swab, a sample of fluid was obtained from the posterior vaginal fornix and rolled onto a prenumbered clean glass slide. The smear was air dried and heat fixed. The slides were Gram stained and examined for budding yeast cells, pseudohyphae and bacteria of various morphotypes, and scored using the Nugent criteria.[5] Nugent scores of 0 - 3 were graded as normal flora, 4 - 6 as intermediate abnormal flora, and 7 - 10 as bacterial vaginosis.

Women were diagnosed as having abnormal vaginal flora if the Nugent criteria for bacterial vaginosis (score 7 - 10) were fulfilled on evaluation of the Gram stain or if budding yeast cells or pseudohyphae suggestive of *Candida* infection were present. To evaluate the role of treatment of abnormal vaginal flora in reducing the risk of perinatal morbidity, the patients with abnormal vaginal flora were randomised into an intervention group and a control group using a computer program. They were asked to attend for follow-up after 2 weeks. Those in the intervention group received vaginal pessaries (clindamycin plus clotrimazole, 100 mg each; Clingen, Aristo Pharmaceuticals, India) to be used once daily for 7 days, while those in the control group did not receive any intervention. Patients were not checked for the effectiveness of the treatment, as many of them were not willing to return for frequent visits.

All the patients received routine antenatal care and were seen for checkups at regular intervals. They were followed up until delivery, and maternal and fetal outcomes were noted. Patients who were diagnosed with any medical disorder (i.e. gestational diabetes, hypertension, severe hypothyroidism) after enrolment or developed any risk factor for preterm delivery were excluded during the course of the study (Fig. 1). The personnel providing the routine antenatal care and conducting the deliveries were blinded to the intervention.

The primary maternal outcome measure was the period of gestation at time of delivery (in completed weeks) and the frequency of late miscarriages (20 - 27 weeks' gestation) and preterm deliveries (28 - 36 weeks' gestation). Secondary outcome measures were the frequency of PROM, the timing of rupture of the membranes (whether intrapartum or antepartum), and infection-related morbidity. PROM was diagnosed by clinical assessment of liquor draining through the cervical os and by the fern test. Postpartum fever is defined by the Joint Committee on Maternal Welfare as an oral temperature of ≥38.0°C on any 2 of the first 10 days after delivery, exclusive of the first 24 hours. The patients were told to take their temperatures twice a day or if they suspected that they had a fever, and to report to us if it was more than 38.0°C.

Statistical analysis

Descriptive statistics were used to analyse the demographic data for age and findings

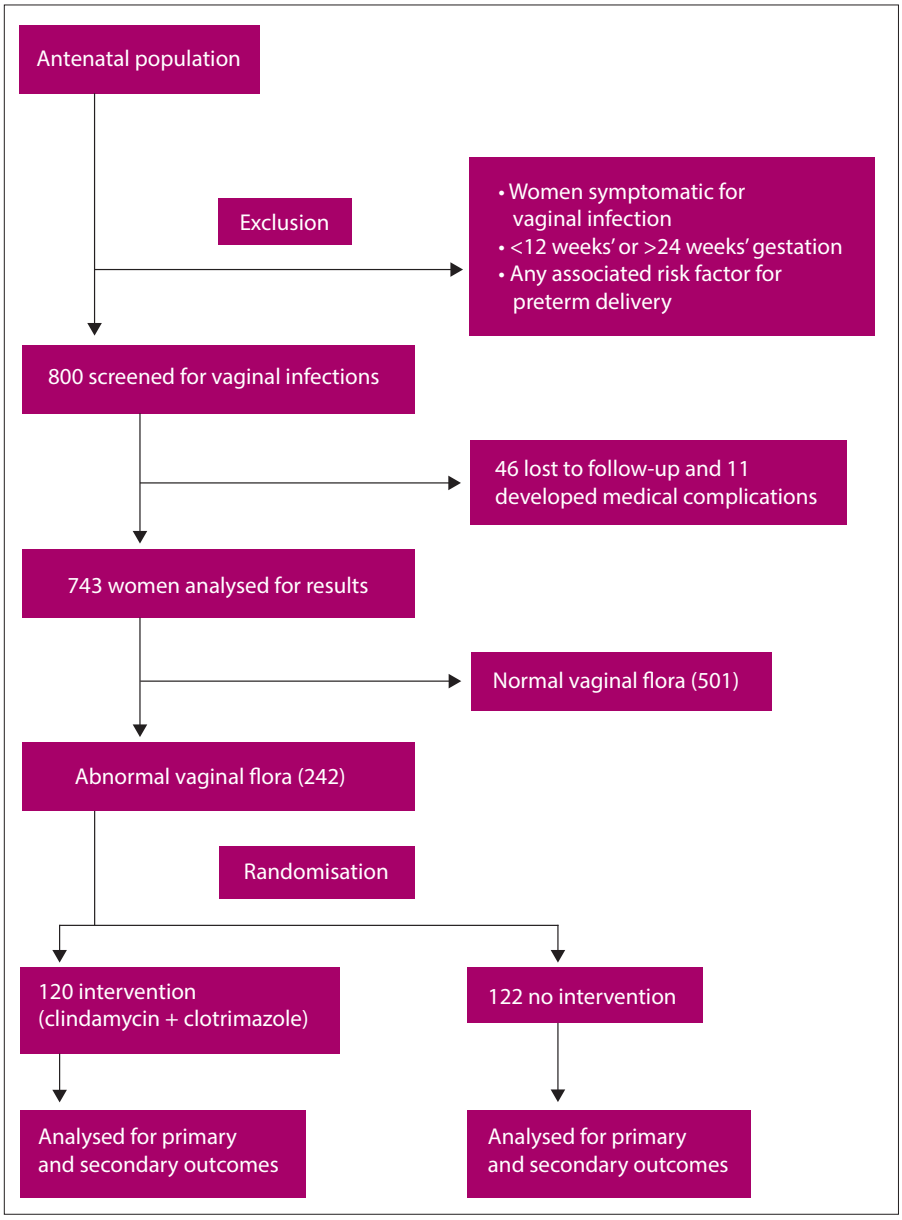

Fig. 1. Trial profile.

on examination. Data to compare the association between various parameters, relative risk (RR), odds ratio and 95% CI were stastically analysed using 2×2 tables and standard formulae. Pearson's chi-square test was used to determine the *p*-value, which was considered significant at <0.05.

Results

Between October 2005 and March 2007 a total of 800 women were recruited into the study. Of these, 743 completed the screening programme (11 patients were excluded due to development of medical disorders and 46 were lost to follow-up) (Fig. 1). The characteristics of the population studied are set out in Table 1.

The presence of abnormal vaginal flora was associated with statistically significant increases in the rate of late miscarriage and preterm delivery. Increases in the rates of PROM and puerperal sepsis were not found to be statistically significant (Table 2).

Maternal outcomes in the intervention and control groups are shown in Table 3. The frequency of preterm birth was as high as 13.6% in the women with normal vaginal flora (Table 2). The overall rate of preterm delivery in the total population was 17.2%. Among patients with vaginal infection, the rate of preterm delivery (gestational age <34 weeks) was 18.6% in the intervention group as opposed to 30.3% in the control group. This decrease was statistically significant (RR 1.65; 95% CI 1.04 - 2.63; $p<0.05$), but decreases in the rates of late miscarriage, PROM and puerperal pyrexia in patients with abnormal vaginal flora who were given treatment were not significant ($p>0.05$).

Discussion

This study showed that the treatment of asymptomatic abnormal vaginal flora in a low-risk population reduced the rate of spontaneous preterm delivery, which was 18.6% in the intervention group as opposed to 30.3% in the control group ($p<0.05$).

Some earlier studies[6-9] did not find a significant decrease in the rate of preterm delivery after the use of antibiotics. In one of these studies,[6] more than half of the patients were randomised after 20 weeks' gestation and the time between diagnosis and initiation

Table 1. Characteristics of the total population

		Abnormal vaginal flora	
Characteristics	**Normal vaginal flora**	**No intervention**	**Intervention**
Age (years), mean±SD	22.38±3.32	23.12±4.2	24.1±3.91
Primigravidas, %	58.1	56.8	59.2
GA at recruitment (weeks), mean±SD	17.6±3.2	19.1±2.6	18.7±3.9
Vaginal infections, %			
BV	-	77.2	74.1
BV + candidiasis	-	1.4	2.1
Candidiasis	-	21.4	23.8

SD = standard deviation; GA = gestational age; BV = bacterial vaginosis.

Table 2. Adverse outcomes in the total population

Outcome	**Normal vaginal flora (*N*=501)**	**Abnormal vaginal flora (*N*=242)**	***p*-value**
Late miscarriage (20 - 27 weeks), *n* (%)	7 (1.4)	22 (9.09)	<0.05
Preterm delivery (28 - 36 weeks), *n* (%)	68 (13.6)	59 (24.3)	<0.05
PROM, *n* (%)	13 (2.6)	22 (9.09)	>0.05
Puerperal pyrexia, *n* (%)	4 (0.7)	9 (3.7)	>0.05

PROM = premature rupture of the membranes.

Table 3. Adverse outcomes after treatment with vaginal clindamycin and clotrimazole

Outcome	**Intervention (*N*=120)**	**No intervention (*N*=122)**	***p*-value**
Late miscarriage: 20 - 28 weeks, *n* (%)	9 (7.5)	13 (10.65)	>0.05
Preterm delivery			
28 - 36 weeks, *n* (%)	22 (18.6)	37 (30.3)	<0.05
30 - 33 weeks, *n* (%)	2 (1.8)	9 (8.2)	<0.05
34 - 36 weeks, *n* (%)	20 (18)	28 (25.6)	>0.05
PROM, *n* (%)	10 (8.3)	12 (9.5)	>0.05
Puerperal pyrexia, *n* (%)	4 (3.3)	5 (4.09)	>0.05

PROM = premature rupture of the membranes.

of treatment was around 8 weeks. This could mean that the upper genital tract was already colonised by the time treatment was instituted at 28 weeks, rendering the treatment ineffective. In our study treatment was started within 2 weeks after randomisation, i.e. by a mean of 20 weeks, resulting in a significant decrease in the rate of preterm delivery.

The treatment we provided differed from from those in other studies[6,10,11] in which oral metronidazole or vaginal clindamycin alone was used. Metronidazole is thought to be inactive against many of the aerobic and fastidious organisms associated with bacterial vaginosis. The intravaginal clindamycin and clotrimazole combination we used may be more effective than oral metronidazole, as it is more active against bacterial vaginosis and has the added benefit of being active against *Candida*, which is a significant cause of morbidity.

In contrast, decreases in the rate of preterm delivery after intervention have also been reported by other researchers.[12-14] Lamont *et al.*[13] reported a significant decrease in the incidence of preterm birth (4% v. 10%) with the use of vaginal clindamycin 2% cream, Ugwumadu *et al.*[12] a significant decrease (5.3% v. 15.7%) with oral clindamycin, and Kiss *et al.*[14] a significant decrease (3% v. 5.3%) with vaginal clindamycin 2% cream. Treatment in these studies was initiated early in pregnancy, with mean gestational ages at screening of 16 - 17 weeks and maximum ages of 19 - 22 weeks. In two of these studies,[12,13] patients were rescreened at the second visit between 24 and 27 weeks and treated if infection persisted. The third study[14] differed in that it included trichomoniasis and candidiasis in the screening programme, and these patients were treated separately and included in the analysis (Table 4).

Cochrane reviews[15,16] have also concluded that treatment of abnormal vaginal flora significantly reduces the rate of preterm birth. These reviews included trials in which patients were treated before 20 weeks' gestation. We noted a significant decrease in the rate of preterm birth even when patients up to 24 weeks' gestation were included. This extends the window of opportunity to treat, which is important in countries like India, where women often first attend for antenatal care relatively late in pregnancy.

Our reduction in the rate of deliveries at less than 34 weeks is also important, as survival and long-term complication rates are worse in infants born before 34 weeks' gestation compared with those born after 34 weeks.

We conclude that simple screening for abnormal vaginal flora in asymptomatic pregnant women and treatment of those affected is an effective and low-cost way of decreasing preterm deliveries and births of low-birth-weight infants. It could have far-reaching consequences in terms of preventing the poor perinatal and long-term outcomes associated with prematurity.

Limitations of our study are the relatively high rate of preterm delivery in the total population studied, and the fact that first-trimester scans were often not available for dating, so in many cases the period of gestation had to be calculated by the LMP or second-trimester scans, which are not very accurate.

Conflicts of interest. There is no conflict of interest.

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Table 4. Comparison of rates of preterm birth after intervention in various studies

Study	Gestational age at screening (weeks), max./mean	Abnormal flora	Intervention, route	Preterm birth (%), intervention v. control	*p*-value
Lamont *et al.*, 2003[13]	20/16	BV	Clindamycin, vaginal	4 v. 10	0.03
Ugwumadu *et al.*, 2003[12]	22/16	BV	Clindamycin, oral	5.3 v. 15.7	0.0003
Kiss *et al.*, 2004[14]	19/17	BV	Clindamycin, vaginal	3 v. 5.3	0.0001
		TV	Metronidazole, oral		
		Candida	Clotrimazole, vaginal		
Present study, 2007	24/19.6	BV, *Candida*	Clindamycin + clotrimazole 100 mg each, vaginal	18.6 v. 30.3	0.003

BV = bacterial vaginosis; TV = *Trichomonas vaginalis.*