PRETERM LABOUR — IS BACTERIAL VAGINOSIS INVOLVED?

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Objective. To assess the efficacy of treatment of bacterial vaginosis (BV) using metronidazole to reduce preterm labour in primigravidae and multigravidae with previous midtrimester abortion or preterm labour.

Design. Randomised controlled trial.

Setting. Tertiary academic hospital.

Method. Two different groups of patients were screened for BV at the first antenatal visit, namely primigravidae and high-risk multigravidae who had had a previous midtrimester abortion or preterm delivery. Patients where BV was diagnosed clinically or on Gram's stain of a smear taken from the posterior vaginal fornix, received either 400 mg metronidazole, or 100 mg vitamin C orally twice daily for 2 days. The Gram's stain was repeated after 4 weeks. If BV was found again, treatment with the same drug was repeated.

Outcome measures. Preterm delivery, birth weight and perinatal deaths.

Results. One thousand and five patients entered the study, but 40 were excluded for various reasons and 10 were lost to follow-up. There were 464 primigravidae, of whom 150 (32%) had BV. Except for the 5-minute Apgar score, no significant differences were found between primigravidae negative for BV and those who received either metronidazole or vitamin C. There were 491 high-risk multigravidae, of whom 127 (26%) had BV. The mean gestational age in the BV-negative group was 37 weeks, in contrast to 37.4 weeks in the vitamin C group and 35.6 weeks in the metronidazole group. Birth weights in these three groups were 2 752 g, 2 759 g and 2 475 g respectively, significantly less ($P = 0.0109$) in the metronidazole group in comparison with the BV-negative group. Delivery before 37 weeks occurred in 29% of high-risk multigravidae with no BV but in 24% of those who took vitamin C and in 43% who took metronidazole. Differences were significant between the BV-negative and metronidazole groups ($P = 0.0231$) and also between the metronidazole and vitamin C groups ($P = 0.0274$). Delivery before 28 weeks occurred in 4% of the high-risk multigravidae with no BV but in 10% of those with BV who took metronidazole. The difference was significant ($P = 0.0430$). Analysis for maximum likelihood estimates for preterm labour identified only previous preterm labour or midtrimester abortion as risk factors.

Conclusion. Metronidazole does not seem to reduce the prevalence of preterm labour when given for BV before 26 weeks' gestation.

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Preterm labour is the major cause of perinatal mortality at Tygerberg Hospital[1] and a common indication for expensive neonatal intensive care.[2] Preterm labour is also a major cause of neonatal handicap. It has been found that 11 - 67% of newborns who weighed less than 800 g and survived the neonatal period, have major neurological sequelae.[3] The prevalence of preterm delivery, according to a community-based study in the Tygerberg area, is 20.3% (Tygerberg Hospital — unpublished data). This is very high indeed when compared with the 4 - 9.3% prevalence in developed countries.[4] It is therefore essential to try to reduce the high prevalence of preterm labour.

Several studies have demonstrated the association between bacterial vaginosis (BV) and preterm labour.[5-10] In addition, some intervention studies have demonstrated a reduction of preterm labour by treating BV.[11,12] The present study was undertaken to determine whether a reduction in the prevalence of preterm labour could also be achieved in a developing country by treating BV.

METHODS

From May 1995 to December 1996, 1 005 primigravidae or mothers who had had a previous preterm labour or midtrimester miscarriage were recruited at the first antenatal visit. Duration of pregnancy at entry to the study was limited to between 15 and 26 weeks' gestation. Patients with multiple pregnancies or known cervical incompetence were excluded from the study. Patients who gave a history of taking any antibiotics within the previous 2 weeks were not immediately enrolled but were seen 2 weeks after they had completed the course, providing they were still less than 26 weeks pregnant.

After informed consent was obtained for the study, a general examination was done followed by a speculum examination. The appearance of the vaginal discharge, if any, was noted and the pH of the vagina was measured. Using an Ayre spatula, a posterior fornix smear was taken for a Gram's stain, wet mount

smear and amine test. For the latter a 10% potassium hydroxide solution was used.[13] The wet mount smear was immediately evaluated for prevalence of lactobacilli, clue cells, *Candida albicans* and *Trichomonas vaginalis*. The other slide was flame-dried, Gram-stained (using carbol fuchsin as counter stain) and immediately examined for lactobacilli, clue cells, *C. albicans* and *Mobiluncus* spp. The presence of lactobacilli, and clue cells was scored as described by Spiegel *et al.*[14] BV was diagnosed if three or more of the following five criteria were present: grey homogeneous discharge, pH > 4.7, positive amine test, 20% or more clue cells, and lactobacilli ≤ 2 +. All wet mount smears and Gram's stains were examined by the same person (JS).

Once BV was diagnosed, the patient was randomised to receive either metronidazole 400 mg twice daily for 2 days, or vitamin C 100 mg twice daily for 2 days. Metronidazole was given for only 2 days as this duration of therapy has been demonstrated to be effective in the treatment of BV.[15] A previous meta-analysis[16] has confirmed the safety of metronidazole in pregnancy. Vitamin C was used for a placebo since the manufacturers could not supply a placebo identical to metronidazole. All tablets were kept in duplicated, numbered sealed opaque envelopes. Selection was done by picking the next envelope from a box. Computer-generated numbers were used to determine the sequence of vitamin C or metronidazole tablets. A balanced block system of envelopes for each group was used to enable possible comparison should it become necessary during the course of the study.

Ultrasound examinations were done to confirm the gestational age when the estimated gestational age was less than 24 weeks, to locate the placenta, measure the cervical length and exclude beaking of the internal cervical os. The biparietal diameter was used to determine the gestational age or, after 24 weeks, the last normal menstruation or clinical estimation of the fundal height.

After 4 weeks, patients who had BV at the first examination had repeat vaginal smears to assess response to therapy. If BV was found again, another 2-day course of the same drug, as given previously, was prescribed, using the remaining envelope with the same number. Patients were followed up for the rest of the pregnancy, labour and puerperium.

At the end of the study, chi-square tests with Yates's correction were used to test the significance of the differences between the different groups. Continuous variables were compared with Student's *t*-tests using the Statgraphics and Epi 6 statistical software program.

RESULTS

A total of 1 005 patients were screened for BV. Before final analysis, 40 mothers (0.4%) were excluded because they failed to meet the inclusion criteria. The reasons for the 19 exclusions in the group without BV were: no previous preterm labour (3), cervical cerclage (1), entered too late (9), and entered too early (6). The 21 exclusions in the group with BV were for cervical cerclage (2), too-early entry (6), cervical pathology (1), prescriptions of metronidazole later in pregnancy (11), and private patient (1). Ten (0.1%) were lost to follow-up. There were 464 primigravidae, of whom 150 (32%) had BV, and 491 multigravidae of whom 127 (26%) had BV (Fig. 1).

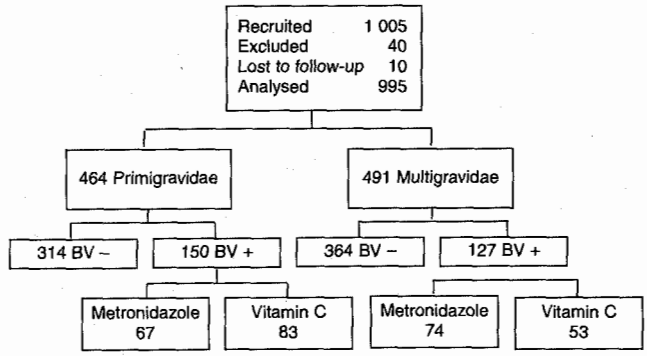

Fig. 1. Trial profile.

Sixty-seven primigravidae received metronidazole and 83 vitamin C as placebo. When the negative, metronidazole and placebo groups were compared, there were no significant differences (Table I). When pregnancy outcome was compared, there were no significant differences except for the lower 5-minute Apgar score in the placebo group than in the BV-positive group (Table II).

Seventy-four multigravidae received metronidazole and 53 placebo. When compared with the BV-negative group, the only significant differences found were a lower maternal age, more

Table I. Clinical data for primigravidae

		Positive for BV		
	Negative for BV	Metro-nidazole	Placebo	*P*-value
N	314	67	83	
Age (yrs)				
Mean	21.3	21.2	22.3	NS
SD	5.2	5.0	4.9	
Cigarette smoking (%)	124 (40)	33 (49)	32 (39)	NS
Antibiotic use (%)	20 (6)	6 (9)	5 (6)	NS
Asymptomatic bacteriuria (%)	35 (11)	7 (11)	11 (13)	NS
Married (%)	45 (14)	7 (11)	13 (16)	NS
Gestation at admission (wks)				
Mean	20.2	20.2	20.4	NS
SD	2.8	3.1	3.0	

BV = bacterial vaginosis.

Table II. Pregnancy outcome in primigravidae

	Negative for BV	Positivefor BV Metro–nidazole	Placebo	*P*-value
N	311*	66	82	
Gestation at delivery (wks)				
Mean	38	38.3	38.4	NS
SD	2.7	2.6	3.0	
Birth weight (g)				
Mean	2 916	2 989	2 942	NS
SD	583	536	616	
5-minute Apgar score				
Mean	9.5†	9.5	9.1†	0.0169†
SD	1.3	0.9	1.7	
< 37 weeks (%)	64 (21)	12 (18)	13 (16)	NS
< 34 weeks (%)	21 (7)	2 (3)	4 (5)	NS
< 28 weeks (%)	2 (0.6)	1 (1.5)	2 (2.4)	NS
IUD (%)	5 (1.6)	-	2 (2.4)	NS
NND (%)	2 (0.6)	1 (1.5)	-	NS
PND (%)	7 (2.3)	1 (1.5)	2 (2.4)	NS

* Two mothers had miscarriages and one patient died before she delivered.
† Significant differences. BV = bacterial vaginosis; IUD = intra-uterine death; NND = neonatal death; PND = perinatal death.

Table III. Clinical data for multigravidae

	Negative for BV	Positive for BV Metro–nidazole	Placebo	*P*-value
N	364	74	53	
Age (yrs)				
Mean	28.4†	27.1†	27.9	0.0426†
SD	5.4	4.5	5.4	
Cigarette smoking (%)	151 (42)	35 (47)	21 (40)	NS
Antibiotic use (%)	13 (4)†	7 (10)†	3 (6)	0.0364†
Asymptomatic bacteriuria (%)	13 (4)†	8 (11)†	4 (8)	0.0147†
Married (%)	207 (57)	38 (51)	23 (43)	NS
Gestation at admission				
Mean (wks)	19.7	19.4	19.9	NS
SD	3.0	3.1	3.1	

† Significant differences.
BV = bacterial vaginosis.

antenatal antibiotic use and more cases of asymptomatic bacteruria in the metronidazole group than in the BV-negative group (Table III). When pregnancy outcome was compared, there was a shorter gestational age, lower mean birth weight and more preterm deliveries in the metronidazole group than in the BV-negative group and a shorter gestational age in the metronidazole group than in the vitamin C group (Table IV). The number of cases in the multigravida and primigravida groups differed because randomisation was not done

Table IV. Pregnancy outcome in multigravidae

	Negative for BV	Positive for BV Metro–nidazole	Placebo	*P*-value
N	351	70	51	
Gestation at delivery				
Mean (wks)	37.0*	35.6*†	37.4†	0.0058*
SD	3.5	4.7	3.2	0.0180†
Birth weight				
Mean (g)	2 752*	2 475*	2 759	0.0109*
SD	792	980	683	
5-Minute Apgar score	9.1*	8.4*	9.4	0.0309*
SD	2.3	2.9	1.5	0.02886†
< 37 weeks (%)	102 (29)*	30 (43)*†	12 (24)†	0.0231* 0.0274†
< 34 weeks (%)	42 (12)*	17 (24)*	6 (12)	0.0067*
< 28 weeks (%)	14 (4)*	7 (10)*	1 (2)	0.0430*
IUD (%)	12 (3.4)	4 (5.7)	1 (1.96)	NS
NND (%)	6 (1.7)	3 (4.3)	-	NS
PND (%)	18 (5.1)	7 (10)	1 (2)	NS

*,†Significant differences.
BV = bacterial vaginosis; IUD = intra-uterine death; NND = neonatal death; PND = perinatal death.

separately for the two groups.

Metronidazole was given to 141 women. At the second visit, attended by 128 women, 4 weeks later, BV was still found in 39 cases (30%). The no-response rate was 31% for primigravidae and 30% for multigravidae. Placebo was prescribed to 136 mothers, of whom 127 attended the second visit. BV was still present in 90 (71%); no response was found in 70% of primigravidae and 72% of multigravidae.

Analysis of maximum likelihood estimates for preterm labour demonstrated an odds ratio of 2.7 for history of a previous preterm labour and 2.0 for previous midtrimester abortion. No other risk factors could be found (Table V).

Discussion

Contrary to the findings of many previous studies,[5-10,17] the frequency of preterm labour was not lower in primigravidae or multigravidae where BV was treated with metronidazole. The three groups of primigravidae were very comparable. As far as the multigravidae are concerned, the metronidazole and vitamin C groups were comparable but the BV-negative group had a lower frequency of antibiotic use and asymptomatic bacteruria. However, it is unlikely that this could have influenced the comparison between the metronidazole and vitamin C groups. The similar outcome of labour in all three groups demonstrates that, in our population, BV or its treatment with metronidazole had no effect on the frequency of

Table V. Analysis for maximum likelihood estimates for preterm labour

Risk factor	*P*-value	Odds ratio
Previous preterm labour	0.0001	2.7
Previous midtrimester abortion	0.0048	2.0
Previous intra-uterine death	0.68	1.1
Alcohol use	0.11	1.6
Cigarette smoking	0.91	1.0
Antibiotic use	0.59	1.0
Positive RPR	0.47	1.0
Asymptomatic bacteriuria	0.96	1.0
Vaginal discharge	0.24	1.4
Positive 'whiff' test	0.86	1.0
pH < 4.5	0.47	1.0
Gram's stain for bacterial vaginosis	0.34	1.0
Presence of *Lactobacillus*	0.35	1.0
Metronidazole treatment	0.22	1.48
Vitamin C	0.31	0.68

RPR = rapid plasma reagin.

preterm labour. The lower mean Apgar score in the placebo group could be regarded as a type I statistical fault which has no clinical significance.

It was quite surprising that preterm labour occurred more frequently in patients where the BV was treated with metronidazole than in the control group where 100 mg vitamin C was used as placebo. This could mean that metronidazole could do harm, or that vitamin C could reduce the risk of preterm labour. However, it is unlikely that such a low dose of vitamin C could have such a marked effect. It is not likely that metronidazole failed to reduce preterm labour because of poor response of BV on the treatment, as the organism was found in only 30% of women after the first treatment in contrast to 71% after vitamin C therapy.

No report could be found in the literature indicating that treatment with metronidazole increases the frequency of preterm labour. However, in a recent study of 913 pregnant women, the 244 cases of BV received metronidazole, metronidazole and erythromycin, or a placebo (J de Souza *et al.* — unpublished data). The prevalence of preterm labour in women with no BV was 16.9%, in women who received the placebo 17.1%, in the metronidazole-alone group 23.5%, and in the erythromycin-metronidazole combination, 14.5%. Women who received only metronidazole had a higher frequency of preterm labour. This is in contrast to the finding of Hauth *et al.*[18] who found that treatment of BV with erythromycin and metronidazole reduced the rates of preterm labour.

Vermeulen and Bruinse[19] treated pregnant women with a history of spontaneous preterm delivery in the preceding pregnancy with either 2% clindamycin vaginal cream or placebo cream. In women who completed the trial, there were significantly more deliveries before 34 weeks' gestation (9% v. 1.4%) in the clindamycin group. Findings of this study are of concern as they demonstrate that poorly indicated treatment may lead to more preterm labour and neonatal morbidity, probably by disturbing the protective effect of normal vaginal flora.

No studies could be found where vitamin C, especially in such a low dose and for such a short time, reduced the frequency of preterm labour. However, it has been found that low ascorbic acid levels were associated with prelabour rupture of membranes, but this was thought to be because of weaker membranes.[20]

Findings of this study question the use of metronidazole for BV in multigravidae at risk for preterm labour.

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