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Effect of Intravaginal Clindamycin Cream on Pregnancy Outcome and on Abnormal Vaginal Microbial Flora of Pregnant Women

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Abstract

Objectives: To determine whether intravaginal clindamycin cream reduces the incidence of abnormal pregnancy outcome in women with abnormal vaginal microbial flora graded as intermediate or BV and to investigate the effect of the antibiotic on vaginal microbial flora.

Methods: A prospective cohort study of pregnant women in an antenatal clinic of a district general hospital. The subjects were 268 women who had abnormal vaginal microbial flora at first clinic visit by examination of a Gram-stained vaginal smear and 34 women with a normal vaginal flora. Two hundred and thirty-seven women were evaluable. Women with abnormal Gram-stained smears (graded as II or III) on clinic recall were randomised to receive treatment (intravaginal clindamycin cream) or placebo and followed to assess outcome of pregnancy, vaginal flora, and detection of *Mycoplasma hominis* and *Ureaplasma urealyticum* after treatment.

Results: Abnormal outcomes of pregnancy were not significantly different in treated and placebo groups by Chi square ($P = 0.2$). However, women with grade III flora responded better to clindamycin than women with grade II flora by numbers of abnormal outcomes ($P = 0.03$) and return to normal vaginal flora ($P = 0.01$) (logistic regression analysis model). This may be due to differences in vaginal bacterial species in these grades. Women whose abnormal vaginal flora had spontaneously returned to normal on follow-up and were therefore not treated (revertants) had as many abnormal outcomes as placebos suggesting that damage by abnormal bacterial species occurred early in pregnancy.

Conclusions: Gram-stain screening distinguishing grade II from grade III flora may be helpful in prescribing treatment other than clindamycin for women with grade II flora. Earlier diagnosis and treatment may be more effective in preventing an abnormal outcome, possibly as soon as pregnancy is diagnosed or even offered as a pre-conception screen. Infect. Dis. Obstet. Gynecol. 8:158–165, 2000.

Key words

bacterial vaginosis; Gram stain and grade of vaginal flora; abnormal outcome of pregnancy; topical clindamycin

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Clinical Study

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Preterm delivery is the most important cause of perinatal mortality and morbidity. Abnormal microbial colonisation of the vagina with a mixture of aerobic, anaerobic, and microaerophilic bacteria, as seen in bacterial vaginosis (BV), is considered to be an important indicator of women at risk.[1–4] In a recent study of pregnant women by our group, those who had BV at their first antenatal clinic attendance were five times more likely to have a preterm delivery or second trimester spontaneous abortion than women who did not have BV.[5] There also was a separate association of grade II (intermediate) flora with late miscarriage.[5] The logical consequence of this was to determine whether antibiotic treatment is effective in reducing the incidence of an abnormal outcome of pregnancy in women with BV and also in women with abnormal flora graded as II and to determine the effect of the antibiotic on the vaginal microbial flora. The results of such a study are presented here.

SUBJECTS AND METHODS

This study was part of a multicenter double-blind placebo-controlled trial to be reported at a later date. The study design for the multicenter study (with a population of 500 subjects: 250 in each arm) provided a power of at least 80% for detecting a 10% difference between groups at a significance level of 5%; that is an abnormal event incidence of 8% in the clindamycin group compared with an expected incidence of 18% in the placebo group. This is based on previous data (R. Lamont, personal communication).

This paper describes the portion of the study completed at Northwick Park Hospital, where additional detailed microbiological findings were carried out together with a study of the revertant group and additional analyses that were not undertaken at the other centres taking part in the study.

Women between 12 and 16 weeks of gestation making their first antenatal visit to Northwick Park Hospital were examined for evidence of abnormal vaginal flora. The prevalence of abnormal vaginal flora in the population of women attending this clinic had been found previously to be 15%.[5] The diagnosis of abnormal vaginal flora was based on microscopic examination of a Gram-stained smear of vaginal secretion. Three categories were recognised as previously described:[5] grade I – normal, comprising predominantly lactobacillus morphotypes; grade II – abnormal (intermediate), in which lactobacilli are reduced and mixed with other bacterial morphotypes; grade III – abnormal (BV) with few or no lactobacillus morphotypes and greatly increased numbers of other morphotypes. The Gram-stained slides were examined after the clinic session. Women diagnosed as having abnormal flora (grade II or grade III) were asked to return to the clinic within three weeks to one month of their initial examination at which time they were examined again for evidence of abnormal flora by the same Gram-stain procedure. Women whose Gram-stained smear was still abnormal (grade II or grade III) were randomised to receive active treatment [intravaginal clindamycin cream (2%) (Pharmacia & Upjohn Ltd.)], or placebo. Treatments were assigned using a computerised randomised-block procedure with a block size of 10. Randomisation was effected by means of a computer-generated random code list with patients allocated to the lowest available code number from the list. Treatment was given at between 16 and 20 weeks of gestation. Informed written consent to take part was obtained from the volunteers, the study having received approval by the institutional Ethics Committee. The following swab specimens were obtained from the women and tested as indicated: an endocervical swab for the detection of *Chlamydia trachomatis* by an enzyme immunoassay (Micro-Trak, Syva), an endocervical swab for the isolation of *Neisseria gonorrhoeae*, and a high vaginal swab for the detection of motile *Trichomonas vaginalis* by microscopic examination of a saline wet-mount preparation. The methods have been described fully elsewhere.[6] Women were excluded from the study if there was any evidence of sexually transmitted disease. Further high vaginal swabs were taken to be examined for aerobic, anaerobic, and microaerophilic species including *Mycoplasma hominis* and *Ureaplasma urealyticum*. Details of the vaginal microbiology have been reported previously.[6]

One hundred and fourteen women were asked to apply 5 grams of clindamycin cream (2%) and 113 women were asked to apply a placebo cream intra-vaginally at bedtime on three consecutive days following diagnosis. The composition of treatment and placebo creams was identical, except that the placebo cream did not contain clindamycin. The latter accounted for a small proportion of the overall formulation. The three-day regimen was

chosen based on a previous clinical study by Pharmacia Upjohn Ltd., showing therapeutic equivalence to seven-day topical clindamycin for treatment of BV. This regimen has been approved in several countries (R. Lamont, personal communication). The women were followed-up with three further outpatient visits at week 3 (20–24 days after entering the study), week 6, and finally at 28–34 weeks of gestation. At each visit, a Gram stain evaluation of vaginal secretion was made. If the Gram-stained smear was still abnormal at week three, a seven-day course of further treatment was offered. This could be either active treatment or placebo depending on initial randomisation. Compliance with the study was assessed by asking patients to return tubes of cream after use, whether empty or not, at the next follow up visit. At the final visit a follow-up swab sample was taken for detection of *M. hominis* and *U. urealyticum*. A final assessment of each woman was made 24 hours after delivery (or 48 hours after miscarriage) to provide a pregnancy outcome report which included a record of abnormal events and a neonate report. In this study as opposed to the whole multicenter trial, two further groups of women attending their first antenatal clinic visit at Northwick Park Hospital also were studied in terms of their vaginal microbial flora, previous obstetric history, and outcome of pregnancy. These were, firstly, 41 women who were described as 'revertants' because they had had an abnormal Gram stain result on their first visit but on returning to the clinic their abnormal vaginal flora was found to have resolved spontaneously and the Gram stain scored as grade I. The second group of 34 women was not randomised, but had normal grade I flora on initial and subsequent screening and clinically had no evidence of BV. The full vaginal microbiology of both groups of women has been described previously.[6]

Statistical Method

A one-way analysis of variance with the Bonferroni multiple comparison procedure was carried out to compare the mean ages in the four groups; abnormal flora (treated and placebo), revertants, and those with normal flora. A logistic regression model was calculated to determine whether an abnormal outcome of pregnancy could be predicted from initial grade of vaginal flora and treatment. This analysis also was performed to determine whether the final grade of vaginal flora could be predicted from the initial grade and treatment. All other associations were tested for statistical significance using the Chi squared test with a P value of less than 0.05 indicating a statistically significant difference.

RESULTS

Description of Cohort

Two hundred and sixty-eight women found to have an abnormal vaginal flora at their first antenatal appointment were asked to return to the clinic for follow-up. On return, 41 (15%) women had resolved their abnormal flora spontaneously (revertant group). Of the remaining 227 women with abnormal flora, 114 received clindamycin and 113 placebo. In addition, there were 34 women with a normal vaginal flora on initial screening with no history of BV.

There were 31 exclusions from the treatment group and 28 from the placebo group. These were: no study data (3), no obstetric/gynaecological data (12), failure to use cream (5), *C. trachomatis* positive (5), *T. vaginalis* positive (1), no follow-up data (5), no follow-up Gram-stained smear (28). Thus there were 83 women who were evaluable in the clindamycin group and 85 in the placebo group. In addition, 4 revertants and 2 with normal flora were excluded leaving a total of 237 evaluable women.

The demographic data of the antenatal population are shown in Table 1. There was no significant difference between the women with abnormal flora given treatment and placebo with respect to age, weight, height, race, and gestational age at baseline, history of smoking, alcohol, or substance abuse. Similarly, there was no significant difference between the groups with respect to past medical history, gravidity, parity, or other features of past obstetric history, or history of sexually transmitted infections. Women with normal vaginal flora were significantly older than those in each of the other three groups ($P < 0.02$); there was no significant difference between the other three groups in terms of age. The demographic characteristics of the group of women with abnormal vaginal flora who were treated were not significantly different from those who were given the placebo ($P > 0.05$). There was a larger proportion of women of Afro-Caribbean origin in the group of women with ab-

TABLE 1. Demographic data for women in the clindamycin trial, for the revertant group, and for healthy pregnant women with normal flora

	Women with abnormal flora			
	Treated with clindamycin	Given placebo	Revertants	Women with normal flora
Number of women	83	85	37	32
Mean age ± s.d.	30 ± 4.5	29 ± 5	29 ± 6	33 ± 5
Ethnic origin known	82	83	37	27
Caucasian	45 (55%)	41 (49%)	22 (59%)	17 (63%)
Asian	17 (21%)	19 (23%)	11 (30%)	6 (22%)
Afro-Caribbean	18 (22%)	21 (25%)	3 (8%)	3 (15%)
Other	2 (2%)	2 (2%)	1 (3%)	1 (4%)
Smoker known	79	82	36	32
No	56 (71%)	58 (71%)	27 (75%)	30 (94%)
Yes	20 (25%)	19 (23%)	5 (14%)	1 (3%)
Exsmoker	3 (4%)	5 (6%)	4 (11%)	1 (3%)
History of abnormal pregnancy	23 (28%)	26 (30%)	14 (38%)	16 (50%)
Spontaneous abortion	19	24	14	14
Preterm delivery[a]	4	4	2	2

[a]Some of the women will have had more than one previous abnormal pregnancy.

normal flora than in the revertants ($P = 0.04$). When combining the data from all women with abnormal flora at first visit (that is, treated, placebo, and revertant groups) there was then a significantly higher proportion of smokers in this combined group than in those with normal vaginal flora ($P = 0.03$). Treated, placebo, and revertant groups had similar incidences of a history of abnormal pregnancy in terms of previous spontaneous abortion and/or preterm delivery. The group with normal vaginal flora showed a surprisingly higher incidence ($P = 0.03$).

A normal distribution of blood groups was found among the group of women who had abnormal flora and also among the sub-groups of women with an abnormal outcome of this pregnancy (data not shown).

Outcome of Pregnancy

The outcome of pregnancy in the four groups is shown in Table 2. When women with an abnormal flora (i.e., grade II and grade III) were considered together, the proportion of women with an abnormal outcome of pregnancy in the treated group (19%) was not significantly different from that in the placebo group (27%) ($P = 0.2$), nor in the revertant group (24%). After combining all women with abnormal flora on first visit, i.e., treated, placebo, and revertant groups, and comparing this combined group with the women who had a normal vaginal flora, the proportion of women in the combined group with an abnormal outcome of pregnancy (23%) was very much greater than the proportion in the group with normal flora (3%) ($P = 0.008$). An interesting finding was that the proportion of women in the revertant group (abnormal flora at first visit which then reverted spontaneously to normal by the time they were recalled to clinic) who had an abnormal outcome of pregnancy (24%) was similar to that in the placebo group with abnormal flora who were not treated (27%).

The data were then analysed further in terms of grade of abnormal flora in relation to abnormal outcome (Table 3). There was no significant difference in numbers of abnormal outcomes between treated women with grade II flora (28%) and treated women with grade III flora (17%) ($P = 0.3$) and similarly no significant difference in the placebo group ($P = 0.2$). There was no significant difference in numbers of abnormal outcomes in women with grade II flora who were treated (28%) and those who were not (16%) ($P = 0.4$). The difference between the treated (17%) and placebo group (30%) of women with grade III flora approached significance ($P = 0.072$). However, a logistic regression model demonstrated a significant interaction between the initial grade of vaginal flora and treatment ($P = 0.03$) (i.e., women with grade III flora responded significantly better to clindamycin treatment than did women with grade II flora in terms of the outcome of pregnancy).

TABLE 2. Outcome of pregnancy

	Women with abnormal flora			
	Treated with clindamycin	Given placebo	Revertants	Women with normal flora
Number of women	83	85	37	32
Pregnancy loss <22 weeks	3	3	0	0
Fetal death	2	1	0	0
Preterm birth				
34–37 weeks	5	4	0	0
<34 weeks	1	3	5	0
Low birthweight				
1.5–2.5 kg	5	10	2	1
<1.5 kg	1	2	3	0
Infections of				
Mother	5	6	6	0
Baby	3	3	2	1
Total number women with abnormal outcome of pregnancy	16 (19%)	23 (27%)	9 (24%)	1 (3%)

TABLE 3. Effect of clindamycin on outcome of pregnancy in relation to grade of vaginal flora

	Grade of vaginal flora (at beginning of study)			
	II (37)		III (131)	
	No. women	No. (%) women with abnormal outcome of pregnancy	No. women	No. (%) women with abnormal outcome of pregnancy
Women treated with clindamycin (83)	18	5 (28%)	65	11 (17%)
Women given placebo (85)	19	3 (16%)	66	20 (30%)

Vaginal Microbiology

The effect of clindamycin on the vaginal microbial flora, as assessed by Gram-stain examination, is shown in Table 4. It is clear that while clindamycin was effective in reducing the proportion of women with abnormal flora in the group whose flora graded initially as III ($P < 0.001$), it did not significantly affect that proportion of women whose flora was graded initially as II ($P = 0.6$). The data were then analysed by a logistic regression model to predict final grade of vaginal flora from initial grade and treatment. There was a significant interaction between the initial grade of vaginal flora and treatment ($P = 0.01$) (i.e., women whose flora initially graded as III and were treated, were significantly more likely to have their flora return to normal than those who were graded as II and were treated).

There was still a substantial proportion of women with grade III flora, that is 16 of 65 (25%), treated with clindamycin whose flora remained abnormal at the end of the study. In view of this the data were analysed to determine if the vaginal microbial flora (at the beginning of the study) of women whose Gram-stained smears remained abnormal throughout pregnancy (in both treated and placebo groups) was different from that of women whose Gram-stained smears returned to normal. It appears that anaerobic Gram-negative rods were underrepresented in the group of women whose flora remained abnormal after clindamycin treatment ($P = 0.02$), whereas the distribution of other bacterial species was similar throughout the groups (data not shown).

Of the women with grade II flora for whom detailed microbiology was available, 6 of 15 (40%) in the treated group and 7 of 12 (58%) in the placebo group had flora that remained as grade II. Of these, two in the treated group and one in the placebo group had group B streptococcal infection after delivery (data not shown).

Baseline swab samples (taken at recruitment to the trial) and follow-up swab samples for the detection of *M. hominis* and *U. urealyticum* were taken from 78 women (42 in the treated and 36 in the placebo group). Detection and cure rates for both these organisms are shown in Table 5.

TABLE 4. Effect of clindamycin on vaginal flora as assessed by Gram stain at end of study

	Grade of vaginal flora (at beginning of study)			
	II (37)		III (131)	
	No. women	No. (%) women with abnormal flora at end of study	No. women	No. (%) women with abnormal flora at end of study
Women treated with clindamycin (83)	18	9 (50%)	65	16 (25%)
Women given placebo (85)	19	11 (58%)	66	51 (77%)

TABLE 5. Detection of *Mycoplasma hominis* and *Ureaplasma urealyticum* from women at recruitment to the study and at follow up

	Treated women (42)	Women given placebo (36)
Mycoplasma hominis		
Before treatmet	15 (36%)	17 (47%)
Follow-up	2 (5%)	13 (36%)
Cure rate	13/15 (87%)	4/17 (23%)
Ureaplasma urealyticum		
Before treatment	25 (60%)	26 (72%)
Follow-up	15 (35%)	21 (58%)
Cure rate	10/25 (40%)	5/26 (19%)

The data were analysed further to determine if the demographic characteristics of the group of treated women whose Gram-stained smear remained abnormal at the end of the study were different from those of the group of treated women whose Gram-stained smear was normal at the end of the study. This also was undertaken on data from the placebo group (data not shown). It appears that smokers in the treated group were slightly but not significantly more likely to have an abnormal Gram-stained smear at the end of the study than were non-smokers. The proportion of Afro-Caribbean women in the four groups was similar, indicating that although women of this ethnic group have a higher incidence of BV (Table 1), they respond to treatment or placebo in a similar way to women in the other ethnic groups. Women with an abnormal Gram-stained smear at the end of treatment were more likely to have a history of abnormal pregnancy and an abnormal outcome in the current pregnancy than those whose Gram-stained smear returned to normal. However, none of the differences was significant.

DISCUSSION

A higher incidence of Afro-Caribbean women and of smokers was found in women who had abnormal vaginal flora. This is consistent with previous data obtained for a similar population of women[5] and is in agreement with the findings of other workers.[7,8] It recently has been suggested that ethnic differences can be accounted for by differences in genital hygiene behaviour as an association has been found between bacterial vaginosis, use of bubble bath, or antiseptic solutions, or douching and ethnicity (black Caribbean).[9] A link between certain blood group antigens and susceptibility to some infectious diseases has been proposed.[10] However, there was no indication from the results of this study that women of a particular blood group were more likely to have an abnormal vaginal flora.

The proportion of women who had an abnormal outcome of pregnancy was very high. Overall, the women attending the antenatal clinic at Northwick Park Hospital represent a low risk population with less than 9% of the population as a whole having a preterm birth.[5] However, in the previous study[5] of women having abnormal vaginal flora, this proportion rose to 16%. Thus we feel that the women with abnormal vaginal flora represent a proportion of the population most at risk. The proportion of women who had an abnormal outcome of pregnancy after treatment with clindamycin was not significantly different from the proportion who had an abnormal outcome after being given the placebo, if women with grade II and grade III flora were considered together. However, if considered separately, women with grade III flora responded to clindamycin treatment significantly better than did women with grade II flora, in terms of outcome of pregnancy and return to normal flora. These findings suggest that there are important differences in the vaginal flora of pregnant women graded as II and III and that careful distinction between the two is essential.

A further important finding was that the proportion of women in the "revertant" group who had an

abnormal outcome (24%) was similar to that of women in the placebo group (27%). This suggests that damage may have been caused by the various bacterial species in the genital tract early in pregnancy before a reversion of the vaginal flora to normality. This is consistent with the findings of Gratacos et al.,[11] who also demonstrated that the diagnosis of BV at any point during pregnancy was associated with an increased risk of perinatal complications even if the abnormal flora was found to resolve spontaneously in examinations later in pregnancy. This invites a determination of the value of treatment earlier in pregnancy.

Subsequent to the demonstration by several groups of investigators of the association between BV and preterm birth,[1–5] there have been several clinical trials to determine the effect of treating BV on the outcome of pregnancy. For example, the results of three separate studies[12–14] showed that topical intravaginal clindamycin was effective in eradicating an abnormal vaginal flora in both non-pregnant[12] and pregnant women.[13,14] However, it did not reduce the risks of perinatal morbidity or preterm delivery.[13,14] In these studies, as in ours, treatment was given at 16–27 weeks of gestation. Oral metronidazole and erythromycin given at 23 weeks of gestation both effectively reduced the prevalence of BV in pregnant women and reduced the rate of preterm delivery.[15] Similarly, oral clindamycin also was effective in reducing the rate of preterm delivery.[16] Although topical therapy may be effective in eradicating local abnormal vaginal flora, it may not affect bacteria colonising the upper genital tract and thus oral therapy may be necessary to prevent the adverse effects of bacteria and bacterial products. A recent intervention study using oral metronidazole to prevent preterm delivery in pregnant women with asymptomatic bacterial vaginosis has failed to demonstrate a reduction in preterm delivery in the treatment group.[17] However, it appears that in some cases up to eight weeks elapsed between diagnosis of BV and randomisation. During this period, ascending colonisation of the upper genital tract may have occurred in a proportion of these women causing damage irreversible by subsequent treatment.

In our study, topical clindamycin contributed to the flora returning to normal in women with grade III flora, but women who had Gram-stained smears of grade II at the beginning of the study were less likely to respond to treatment. The full microbiology of the different Gram-stain categories has been described before[6] and it has been shown that the flora can switch spontaneously from an abnormal to a normal state. The data from our previous study[6] suggest that there is a trend for certain bacterial species to be expressed in large numbers in a definite order with anaerobic Gram-negative rods and anaerobic Gram-positive cocci increasing in numbers at the end of grade II and the beginning of grade III, and *Gardnerella vaginalis* and *M. hominis* organisms occurring in large numbers only in the very late stages. As all these organisms are very sensitive to clindamycin, it seems likely that women with grade III flora will respond well to clindamycin (in terms of a return to normal flora). This was shown in this study both with regard to the results of Gram-staining vaginal smears at the end of study and to the detection rate of *M. hominis* after treatment. Interestingly, *U. urealyticum* organisms were little affected by treatment, which is not surprising since they are not sensitive to therapeutic levels of clindamycin. It is a moot point whether their elimination would have improved further the outcome of pregnancy, particularly as 42% of the women with normal vaginal flora in our previous study[6] were positive for *U. urealyticum*, with no abnormal outcome of pregnancy.

The results of our previous study[6] showed that women with grade II flora had a higher incidence of beta-haemolytic *Streptococcus* spp. which are relatively much less sensitive to clindamycin. Furthermore, women whose Gram-stained smear remained abnormal were less likely to be carrying anaerobic Gram-negative rods at the beginning of the study. Thus, the vaginal microbial flora of these women was less responsive to clindamycin therapy. In the other clinical studies referred to,[12–17] no distinction was made between women with flora graded as II or III and therefore it was not possible to identify the sub-group of women potentially unresponsive to clindamycin.

CONCLUSIONS

The results of this study have demonstrated for the first time that a sub-group of women exists within those who have an abnormal vaginal flora and that this sub-group is less likely to respond to topical clindamycin therapy in terms of a return to normal vaginal flora and outcome of pregnancy. The sub-

group comprises mainly women with grade II vaginal flora who can easily be identified by examination of a Gram-stained smear of vaginal secretion early in pregnancy. However, a further group of women whose Gram-stained smears are graded as III, but who are less likely to be carrying anaerobic Gram-negative rods than other women who have grade III smears, also may be less likely to respond to clindamycin. More work is needed to determine what factors (microbiological or otherwise) may be determining abnormal outcome in this sub-group.

Data from the revertant group suggest that damage from abnormal flora may have occurred early in pregnancy and thus earlier diagnosis and treatment may demonstrate a significant reduction in abnormal outcome of pregnancy. We would thus recommend that screening for abnormal flora should be carried out as soon as pregnancy is diagnosed or even offered as a pre-conception screen.

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