Human Reproduction vol 11 no 7 pp 1575–1578, 1996

Association of bacterial vaginosis with a history of second trimester miscarriage*

J.M.Llahí-Camp[1], R.Rai[2], C.Ison[3], L.Regan[2,4] and D.Taylor-Robinson[1]

[1]MRC Sexually Transmitted Diseases Research Group, [2]Department of Obstetrics and Gynaecology, [3]Department of Microbiology, Imperial College School of Medicine at St. Mary's, Paddington, London W2, UK

[4]To whom correspondence should be addressed at: Recurrent Miscarriage Clinic, Samaritan Hospital for Women, Marylebone Road, London NW1 5YE, UK

The aim of this study was to determine whether bacterial vaginosis (BV) is associated with a history of recurrent pregnancy loss. A total of 500 consecutive patients attending the Recurrent Miscarriage Clinic were screened for the presence of BV. In women who had had at least one late miscarriage BV was found twice as commonly (27/130; 21%) as in women who had had only early losses (31/370; 8%) ($P < 0.001$). The difference was even larger (26 versus 8%) if women who had had term pregnancies were excluded. Moreover, BV was found three times more commonly in Afro-Caribbean women [17 (29%) of 58] than in Caucasian women [36 (9%) of 379] and, in both groups of women, BV was diagnosed at least twice as frequently in those with a history of at least one late miscarriage than in those who had experienced first trimester pregnancy losses only ($P < 0.001$). The condition occurred twice as often among smokers than non-smokers and, in both groups, it was at least twice as common in women with a history of at least one late miscarriage as in those who had had early pregnancy losses only ($P < 0.001$). However, the relationship between BV and smoking was independent of ethnic origin. Women who douched with chloroxylenol were mostly Afro-Caribbean and had BV more than twice as often as women who did not douche.

Key words: bacterial vaginosis/ethnic origin/hygiene habit/recurrent miscarriage/smoking

Introduction

Bacterial vaginosis (BV) is a condition characterized by an alteration of the vaginal ecology in which the normal flora, dominated by lactobacilli, is replaced by a mixed bacterial flora which includes *Gardnerella vaginalis, Mobiluncus* spp., *Mycoplasma hominis, Bacteroides* spp. and other anaerobes (Spiegel, 1991). Women with BV have an elevated vaginal pH (>4.5) and can experience a vaginal homogeneous discharge which often has a distinctive fishy odour. The addition of potassium hydroxide to a drop of vaginal secretion on a slide may produce or accentuate the odour and these three signs in addition to the presence of 'clue cells' on a wet mount smear comprise the composite criteria. If any three of the four criteria exist, then a diagnosis of BV may be made (Eschenbach *et al.*, 1984). Alternatively, the diagnosis may be made on the basis of microscopic examination a Gram-stained vaginal smear (Spiegel *et al*, 1983; Nugent *et al*, 1991).

The prevalence of BV in the general British population is in the region of 9% (Morgan *et al.*, 1994). However, the prevalence of BV has been reported to be significantly higher, between 16 and 18%, among women presenting in preterm labour or with premature rupture of membranes (Lamont *et al*, 1986; Martius *et al.*, 1988). The results of prospective studies have shown that women who have BV at the beginning of the second trimester have a 5-fold increase in their risk of having preterm labour or late miscarriage (Gravett *et al.*, 1986; McGregor *et al.*, 1990; Kurki *et al.*, 1992; Hay *et al.*, 1994) and deliver preterm low birth-weight infants independently of other recognized risk factors (Hillier *et al.*, 1995). Although the association between BV and the poor outcome of pregnancy is best defined in such prospective studies, we considered that it would nevertheless be valuable retrospectively to establish whether BV was associated with a history of recurrent pregnancy loss, by examining women attending a dedicated miscarriage clinic.

Materials and methods

Patients

A total of 500 consecutive women (median age 33 years; range 20–47 years) attending the Recurrent Miscarriage Clinic at St Mary's Hospital, London, UK was examined. All women had experienced at least three consecutive miscarriages (median 4; range 3–9) They comprised those who had had early (first trimester, ≤13 completed weeks of gestation) and/or late (second trimester; >13 completed weeks of gestation) miscarriages. None of them was pregnant nor taking any hormonal preparation at the time of the investigation

Procedure

All women gave informed verbal consent to undergo speculum examination. The lateral vaginal fornix was sampled by passing a plastic loop through a non-lubricated vaginal speculum. The specimen was smeared on a glass slide, and the smear fixed immediately in 95% methanol and allowed to dry in air The smear was stained by Gram's method and examined microscopically by one investigator

*This work was partially presented at the Annual Conference of the British Fertility Society, Liverpool, May, 1995 and as a poster in the 11th Annual Meeting of the European Society of Human Reproduction and Embryology, Hamburg, Germany, July, 1995

(J.L -C) within 1 week and reviewed by a second investigator (C.I.) within 1 month.

A diagnosis of BV was made on the basis of the Gram stain, using well established criteria (Spiegel *et al.*, 1983) A smear was graded as I (normal; predominantly *Lactobacillus* morphotypes); II (intermediate; reduced number of *Lactobacillus* morphotypes and the presence of other bacterial morphotypes) or III (BV, absence of *Lactobacillus* morphotypes with greatly increased numbers of other bacterial morphotypes).

Statistical analysis

The prevalence of BV in women with a history of early or late miscarriage was compared using the χ^2 test. The relationship between ethnic origin and BV and miscarriage, and between smoking habit and BV and miscarriage were compared using logistic regressions.

All calculations were performed using the SPSS/PC statistical package on an IBM-compatible computer

Results

Relationship between BV and history of miscarriage irrespective of term pregnancy

Of the 500 women examined, 370 (74%) (median age 33.4 years; range 21–46) had experienced only first trimester (early) miscarriages, and 130 (26%) (median age 33.1 years; range 20–47) had a history of at least one second trimester (late) pregnancy loss.

BV (grade III Gram stain) was found significantly more often among women with a history of at least one late miscarriage (27/130; 21%) than among those with recurrent early pregnancy loss only (31/370; 8%) ($P < 0.001$) (Table I). Grade II smears were also found significantly more often in the late miscarriage group ($P < 0.05$).

Among women with a history of at least one pregnancy loss, those who had had recurrent (two or more) second trimester miscarriages, had BV in a similar proportion (14/67; 21%) to the group as a whole (27/130; 21%).

Relationship between BV and history of miscarriage and term pregnancy

Of 324 women who had never had a term pregnancy, BV was found significantly more often among those with a history of at least one late miscarriage (22/86; 26%) than among those who had had recurrent early pregnancy losses only (19/238; 8%) ($P < 0.001$) (Table II).

Of 176 women who had had a term pregnancy, BV was not found significantly more often among those who had had late rather than early miscarriages (Table II).

Relationship between ethnic origin and BV and miscarriage

One in five Caucasian women had a history of one or more late miscarriages, whereas three in five Afro-Caribbean women had such a history (Table III). The results also show that BV was found three times more commonly in Afro-Caribbean women [17 (29%) of 58] than in Caucasian women [36 (9.4%) of 379)] and that in both groups of women BV was at least twice as common in those with a history of at least one late miscarriage as in those who had experienced first trimester pregnancy losses only ($P < 0.005$).

Table I. Relation of bacterial vaginosis to miscarriage irrespective of term pregnancy

Miscarriage[b]	No (%) of women with indicated Gram stain grading[a]			Total
	Grade I	Grade II	Grade III	
Early (%)	318 (86)	21 (6)	31 (8)	370
Late (%)	90 (69)	13 (10)	27 (21)	130

[a]Grade I = normal, only *Lactobacillus* morphotypes, grade II = intermediate, both *Lactobacillus* and other bacterial morphotypes; grade III = absence of *Lactobacillus*
[b]Early = ≤13 weeks gestation; late = >13 weeks gestation.

Table II. Relation of bacterial vaginosis to history of miscarriage and term pregnancy. A, Women who had never had a term pregnancy (n = 324); B, women with at least one term pregnancy (n = 176)

Miscarriage[b]	No (%) of women with indicated Gram stain grading[a]			Total
	Grade I	Grade II	Grade III	
A				
Early (%)	206 (87)	13 (5)	19 (8)	238
Late (%)	55 (63)	9 (11)	22 (26)	86
B				
Early (%)	112 (85)	8 (6)	12 (9)	132
Late (%)	35 (80)	4 (9)	5 (11)	44

[a,b]See Table I for explanation.

Table III. Relation of ethnic origin to history of miscarriage and to Gram stain grading

Ethnic origin	Miscarriage[b]	No (%) of women with indicated Gram stain grading[a]			Total
		Grade I	Grade II	Grade III	
Caucasian	Early (%)	264 (86)	16 (6)	24 (8)	304 (80)
(n = 379)	Late (%)	55 (73)	8 (11)	12 (16)	75 (20)
Afro-Caribbean	Early	17 (71)	3 (12)	4 (17)	24 (41)
(n = 58)	Late	17 (50)	4 (12)	13 (38)	34 (59)
Asian	Early	17 (94)	1 (6)	0 (0)	18 (69)
(n = 26)	Late	7 (87)	0 (0)	1 (13)	8 (31)
Others	Early	20 (83)	1 (4)	3 (13)	24 (64)
(n = 37)	Late	11 (84)	1 (8)	1 (8)	13 (36)

[a,b]See Table I for explanation

Relationship between smoking habit and BV, miscarriage and ethnic origin

BV occurred twice as commonly among smokers as in non-smokers and in both groups it was at least twice as common in women with a history of at least one late miscarriage as in those who had had early pregnancy losses only ($P < 0.001$) (Table IV). However, the relationship of BV to smoking was independent of ethnic origin because, as shown in Table V, the proportion of Afro-Caribbean women who smoked was lower than that of the Caucasian women who did so.

Table IV Influence of smoking on the occurrence of bacterial vaginosis in women with recurrent miscarriage

Smoking habit	Miscarriage[b]	No (%) of women with indicated Gram stain grading[a]			Total
		Grade I	Grade II	Grade III	
Smoker	Early (%)	63 (77)	6 (7)	13 (16)	82
	Late (%)	14 (58)	3 (12)	7 (30)	24
	Total	77	9	23	106
Non-smoker	Early (%)	242 (89)	14 (5)	17 (6)	273
	Late (%)	69 (73)	10 (11)	15 (16)	94
	Total	311	24	32	367
Unknown	Early	13	1	1	15
	Late	7	0	5	12
	Total	20	1	6	27

[a,b]See Table I for explanation.

Table V Relation of ethnic origin to smoking habit

Ethnic origin	No (%) of women who		
	Smoke	Do not smoke	Unknown
Caucasian (%)	81 (22)	280 (74)	18
Afro-Caribbean	10 (17)	41 (71)	7
Asian	5	20	1
Others	6	26	5

Table VI Relation of hygiene habit to BV in women of various ethnic groups

Use of		No (%) of women with indicated Gram stain grading[a]			Total
		Grade I	Grade II	Grade III	
'Dettol'	Caucasian	0	0	0	0
	Afro-Caribbean (%)	7 (64)	0	4 (36)	11
	Asian	2	0	0	2
	Others	0	0	0	0
Bubble bath	Caucasian (%)	82 (86)	3 (3)	10 (11)	95
	Afro-Caribbean	4	0	2	6
	Asian	6	0	0	6
	Others	1	1	0	2
Soap	Caucasian (%)	34 (85)	2 (5)	4 (10)	40
	Afro-Caribbean	2	1	4	7
	Asian	4	0	0	4
	Others	3	0	0	3
Unknown		263	27	34	324

[a]See Table I for explanation.

Relationship between vaginal douching and BV

Women who used 'bubble bath', most of whom were Caucasian, had BV no more frequently than those who only used soap (Table VI). However, women who douched with 'Dettol' (chloroxylenol, 4.8%), most of whom were Afro-Caribbean, had BV more than twice as often as women who did not douche.

Discussion

In this retrospective study, we chose to use the Gram stain rather than the composite criteria for diagnosing BV because it is simpler, becoming accepted as a means of diagnosis (Hay *et al.*, 1992) and has the virtue of defining grade II flora which does not have a clinical equivalent. On this basis, the proportion of women with a history of early miscarriage who had BV was the same as the proportion of women in the general population who have BV, that is 9% (Morgan *et al.*, 1994). However, BV was found in a significantly larger proportion (21%) of women with a history of at least one late miscarriage ($P < 0.001$). This proportion was found to be even larger (26%) if women who had had term pregnancies were excluded.

Afro-Caribbean women had experienced late miscarriages three times more often than Caucasian women and women with BV were three times more likely to be Afro-Caribbean than Caucasian. This suggests a link between the finding of BV and the occurrence of late miscarriage. Indeed, if BV had not been found to increase proportionally in the Afro-Caribbean women in relation to the increase in miscarriage, but had remained the same as in the Caucasian women, it would not be reasonable to suggest such a link.

The occurrence of BV more frequently in smokers, also noted by Hay *et al.* (1994), was not related to ethnicity. Maternal smoking influences placental enzyme production (Barnea, 1994) and placental hormonal secretion (Shurtz-Swirski *et al.*, 1992) *in vitro*. Greatly enhanced proliferation of bacteria in the murine vagina by oestrogen administration has been demonstrated (Furr *et al.*, 1991), so that, indirectly, smoking might in some way contribute to the aetiology of BV. In relation to the latter, a history of douching with 'Dettol' was associated significantly with the finding of BV and was a procedure undertaken by Afro-Caribbean women who, overall, were found to have BV more often than other women. It is tempting, therefore, to implicate the douching as a contributory factor in the development of BV, but it is unclear whether this is so or whether douching is carried out because of the existence of BV.

BV diagnosed in early pregnancy is undoubtedly associated with preterm labour or late miscarriage (Gravett *et al.*, 1986; McGregor *et al.*, 1990; Kurki *et al.*, 1992; Hay *et al*, 1994) and with delivery of low birth-weight infants (Hillier *et al.*, 1995). There are several theories to explain this relationship. The first maintains that proteases produced by the various bacteria found in BV weaken and destroy the connective tissue framework that makes up the fetal membranes and thus lead to their rupture (McGregor *et al.*, 1989). It has also been suggested that mucinases and sialidases produced by the bacteria hydrolyse the protective cervical mucin so allowing entry of micro-organisms into the uterus where they cause infection and inflammation (Henrichs *et al.*, 1982; Reutter *et al.*, 1982). In support of this theory is the frequent histological evidence of endometritis in non-pregnant women with BV and recovery of bacteria associated with BV from fetal membranes and amniotic fluid subsequent to premature rupture of membranes and preterm labour (Paavonen *et al.*, 1987). Furthermore, prostaglandins as well as endotoxins and interleukin-1a have been found at high concentrations in the cervical mucus and vaginal secretions of pregnant women with BV (Platz-Christensen *et al.*, 1992, 1993).

The results of the study reported here show for the first time that BV is associated with a history of late pregnancy

loss. However, the question arises as to whether BV diagnosed currently has developed subsequent to the miscarriages or whether it is a reflection of long-standing BV that might have been responsible for the pregnancy losses. Apart from the evidence mentioned above in favour of the latter, it is noteworthy that BV is often a recurrent problem and in addition, exists most often asymptomatically. However, to determine whether our establishment of an association between BV and a history of late miscarriage is helpful in foretelling the outcome of a future pregnancy in the same women, we are conducting a prospective longitudinal study to determine the predictive value of a diagnosis of BV before pregnancy and at various stages of gestation, on the outcome of the pregnancy.

Acknowledgement

We thank the nursing staff, especially Indra Jaswal, at the Samaritan Hospital for Women, London NW1, for their help in this study and to Jane Wandsworth for her help with the statistical analyses

References

Barnea, E R (1994) Modulatory effect of maternal serum in xenobiotic metabolizing activity of placental explants modification by cigarette smoking *Hum Reprod.*, **9,** 1017–1021

Eschenbach, D.A., Bekassy, S , Blackwell, A. *et al.* (1984) The diagnosis of bacterial vaginosis. In Mårdh, P.A. and Taylor-Robinson, D. (eds), *Bacterial Vaginosis* Almqvist & Wiksell International, Stockholm, pp 260–261

Furr, P.M. and Taylor-Robinson, D (1991) The influence of hormones on the bacterial flora of the murine vagina and implications for human disease *Microb Ecol. Health Dis* , **4,** 141–148

Gravett, M G., Nelson, H.P., DeRouen T *et al.* (1986) Independent associations of bacterial vaginosis and *Chlamidia trachomatis* infection with adverse pregnancy outcome. *JAMA*, **256,** 1899–903

Hay, P E , Taylor-Robinson, D and Lamont, R F (1992) Diagnosis of bacterial vaginosis in a gynaecology clinic *Br J. Obstet. Gynaecol.*, **99,** 63–66

Hay, P.E , Lamont, R F , Taylor-Robinson, D *et al.* (1994) Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage. *Br. Med J* , **308,** 295–299.

Henrichs, P.A.J., Van Erne-Vandertol, M.E. and Verholef, J (1982) Partial removal of sialic acid enhances phagocytosis and the generation of superoxide and chemiluminescence by polymorphonuclear leukocytes *J. Immunol* , **129,** 745–749.

Hillier, S L., Nugent, R P , Eschenbach, D.A. *et al.* for the Vaginal Infections and Prematurity Study Group (1995) Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant *N. Engl. J Med*, **333,** 1737–1742

Kurki, T., Sivonen, A., Renkonen, O *et al* (1992) Bacterial vaginosis in early pregnancy and pregnancy outcome *Obstet Gynecol.*, **80,** 173–177

Lamont, R F , Taylor-Robinson, D , Newman, M. *et al* (1986) Spontaneous early preterm labour associated with abnormal genital colonization. *Br J Obstet Gynaecol* , **93,** 804–810

Martius, J., Krohn, M , Hillier, S *et al* (1988) Relationships of vaginal *Lactobacillus* species, cervical *Chlamydia trachomatis*, and bacterial vaginosis to preterm birth *Obstet. Gynecol.*, **71,** 89–95

McGregor J.A , French J.I., Lawellin D *et al.* (1989) In vitro study of bacteria protease-induced reduction of chorioamnionitis membrane strength and elasticity. *Obstet Gynecol.*, **69,** 167–174.

McGregor, J.A , French, J.I , Richter, R. *et al.* (1990) Antenatal microbiologic and maternal risk factors associated with prematurity. *Am. J. Obstet Gynecol* , **163,** 1465–1473.

Morgan, D.J , Wilden, S D., Butcher, R. *et al.* (1994) *3rd International Symposium on Vaginitis/Vaginosis*. Madeira, Portugal, January 1994

Nugent, R.P., Krohn, M.A. and Hillier, S.L. (1991) Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation *J Clin. Microbiol.*, **29,** 297–301.

Paavonen, V., Tersala, K. and Heinone, P.K. (1987) Microbiologic and histopathologic findings in acute pelvic inflammatory disease. *Br. J Obstet Gynaecol.*, **94,** 454–460.

Platz-Christensen, J.J., Brandberg, A. and Wiqvist, N (1992) Increased prostaglandin concentration in the cervical mucus of pregnant women with bacterial vaginosis *Prostaglandins*, **43,** 133–134.

Platz-Christensen, J.J., Mattsby-Baltzer, I , Thomsen, P. and Wiqvist, N. (1993) Endotoxin and interleukin-1 alpha in the cervical mucus and vaginal fluid of pregnant women with bacterial vaginosis. *Am. J Obstet Gynecol.*, **169,** 1161–1166

Reutter, W E., Kottgen, E., Baver, C. *et al.* (1982) Biologic significance of sialic acids. In Shaver, R. (ed), *Sialic Acids Chemistry, Metabolism and Function* Springer-Verlag, New York, pp 263–305

Shurtz-Swirski, R , Barnea, E.R., Rosenblum, R. and Check, J H. (1992) Effect of maternal cigarette smoking upon placental hCG secretion *in vivo*. *Assist. Reprod. Technol. Androl* , **3,** 397–403.

Spiegel, C (1991) Bacterial vaginosis *Clin. Microbiol. Rev.*, **4,** 485–502.

Spiegel, C , Amsel, R and Holmes, K (1983) Diagnosis of bacterial vaginosis by direct Gram stain of vaginal fluid. *J Clin. Microbiol.*, **18,** 170–177

Received on February 14, 1996, accepted on April 25, 1996