Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV

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Background: Cross-sectional studies suggest an association between bacterial vaginosis (BV) and HIV-1 infection. However, an assessment of a temporal effect was not possible.

Objectives: To determine the association of BV and other disturbances of vaginal flora with HIV seroconversion among pregnant and postnatal women in Malawi, Africa.

Design: Longitudinal follow-up of pregnant and postpartum women.

Methods: Women attending their first antenatal care visit were screened for HIV after counselling and obtaining informed consent. HIV-seronegative women were enrolled and followed during pregnancy and after delivery. These women were again tested for HIV at delivery and at 6-monthly visits postnatally. Clinical examinations and collection of laboratory specimens (for BV and sexually transmitted diseases) were conducted at screening and at the postnatal 6-monthly visits. The diagnosis of BV was based on clinical criteria. Associations of BV and other risk factors with HIV seroconversion, were examined using contingency tables and multiple logistic regression analyses on antenatal data, and Kaplan–Meier proportional hazards analyses on postnatal data.

Results: Among 1196 HIV-seronegative women who were followed antenatally for a median of 3.4 months, 27 women seroconverted by time of delivery. Postnatally, 97 seroconversions occurred among 1169 seronegative women who were followed for a median of 2.5 years. Bacterial vaginosis was significantly associated with antenatal HIV seroconversion (adjusted odds ratio = 3.7) and postnatal HIV seroconversion (adjusted rate ratio = 2.3). There was a significant trend of increased risk of HIV seroconversion with increasing severity of vaginal disturbance among both antenatal and postnatal women. The approximate attributable risk of BV alone was 23% for antenatal HIV seroconversions and 14% for postnatal seroconversions.

Conclusions: This prospective study suggests that progressively greater disturbances of vaginal flora, increase HIV acquisition during pregnancy and postnatally. The screening and treating of women with BV could restore normal flora and reduce their susceptibility to HIV.

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Keywords: Bacterial vaginosis, HIV incidence, Malawi, seroconversion, sexually transmitted diseases, women

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Introduction

Bacterial vaginosis (BV) is common among African women where prevalence rates of more than 50% have been reported [1,2]. It is characterized by changes in the vaginal flora resulting in loss of lactobacilli, an increase in other predominantly anaerobic flora and an increase in vaginal pH [3]. Hydrogen peroxide (H_2O_2)-producing strains of lactobacilli are found in about 96% of women with normal vaginal microflora, but only in about 6% of women with BV, suggesting an important protective role against BV by the (H_2O_2)-producing lactobacilli [4]. The acidic environment of the normal vaginal flora inhibits colonization by potentially pathogenic bacteria while an elevated pH facilitates the growth of sexually transmitted disease (STD) agents such as *Trichomonas vaginalis* [5–7]. Additionally, a low pH inactivates both free HIV-1 virus and T lymphocytes in vaginal fluid and is less favourable for the growth of HIV and infected lymphocytes [8,9]. Women with higher vaginal pH such as in BV could be more susceptible to HIV infection or be more infectious to their partners due to an increased titre of infectious virus [9].

The association of BV with HIV acquisition or transmission has not been adequately investigated. However, a recent cross-sectional study from Uganda [1] found a significant association between HIV prevalence and BV among younger women, but not among women older than 40 years. This association was not related to differences in sexual activity or concurrent STDs. The authors suggested that abnormal vaginal flora could increase HIV acquisition since HIV infection among young women is likely to be recent. This paper examines in a longitudinal study, the association of BV and other disturbances of vaginal flora with acquisition of HIV among pregnant and postpartum women.

Methods

Two large cohorts of pregnant women were recruited in 1990 and 1993 at the Queen Elizabeth Central Hospital, a tertiary care facility, in Blantyre, Malawi (south-east Africa), to determine the incidence of HIV and to study the risk factors associated with HIV seroconversion. After appropriate counselling and obtaining informed consent, women were screened for HIV and other STDs at their first antenatal visit during pregnancy. HIV-seronegative women were enrolled in a prospective study and followed during pregnancy and postpartum. In addition to consent, the inclusion criteria for enrolment were being a resident of Blantyre city or its immediate suburban townships, willingness to deliver in the hospital and to return for follow-up visits. Serum HIV testing of seronegative women to confirm seroconversion was performed at delivery and at 6-monthly follow-up visits postpartum. Pelvic examinations and collection of laboratory specimens to determine disturbances of vaginal flora and STDs were done at screening and at the 6-monthly postpartum visits. Social, behavioural and demographic data related to HIV transmission were collected on all women at screening. Treatment of STDs (gonorrhea, syphilis, trichomoniasis and candidiasis) and condoms were available in the clinic at no cost.

HIV results were based on enzyme immunoassay (EIA) and Western blot tests. In the 1990 cohort, all women that were positive on a repeat EIA (Wellcozyme; Wellcome Diagnostics, Dartford, Kent, UK) were confirmed by a Western blot test (Bio-Rad, Hercules, California, USA). In the 1993 cohort, the women were screened using two independent EIA tests (Wellcozyme and Behring, Marburg, Germany) and only borderline specimens were confirmed by a Western blot test. Seroconversions in both cohorts, were confirmed by a Western blot test following two EIA tests. Syphilis testing was carried out using the Rapid Plasma Reagin test (Macro-Vue, Becton-Dickinson, Cockysville, Maryland, USA) for screening and FTA or TPHA (Sera-Tek, Miles Inc., Elkhart, Indiana, USA) tests for confirmation. Vaginal wet mounts were examined for clue cells, motile trichomonads and yeast cells. Cervical swabs to isolate *Neisseria gonorrhoeae* were smeared on modified Thayer–Martin media, stored instantly in CO_2 extinction jars and transported to the laboratory where, after incubation, positive cultures were determined by colonial morphology and Gram stain.

Disturbances of vaginal flora and BV were based on four criteria: (a) a vaginal pH > 4.5 (measured by pH paper on a vaginal swab obtained from lateral and posterior fornices); (b) an increased homogeneous vaginal discharge (to be more objective, we limited this criterion to a vaginal discharge which was not associated with concurrent gonorrhea, trichomoniasis or candidiasis); (c) presence of clue cells in ≥ 20% of vaginal epithelial cells (detected by mixing vaginal fluid with a drop of normal saline on a slide and examining under high power magnification); and (d) a positive amine or Whiff test (performed by mixing a few drops of 10% potassium hydroxide with vaginal fluid).

The presence of vaginal discharge was determined by a speculum-aided pelvic examination conducted by trained nurses. The other criteria were determined by laboratory technicians who were not aware of the woman's clinical history or the results of her pelvic examination. We classified women with none of the four clinical criteria as having normal vaginal flora.

Women with one or two clinical criteria were classified as having 'disturbance of vaginal flora', a non-specific abnormal vaginal milieu. Women with three or more clinical criteria were classified as having BV which is consistent with the conventional clinical definition of BV [10]; this could be equivalent to a score of 7–10 on a Gram stained vaginal smear [11].

Descriptive, univariate and multivariate analyses were conducted to determine rates of HIV and STD and to assess associations between disturbances of vaginal flora and HIV serconversion. We analysed seroconversion separately during pregnancy and postnatally for two reasons: (a) to assess the role of disturbance of vaginal flora and BV before and after delivery since physiological changes during pregnancy and early postpartum period may influence the prevalence of vaginal microflora [5], and (b) follow-up antenatally, from enrolment to delivery, was complete and was not influenced by losses to follow-up or censoring.

Contingency tables and logistic regression were used to study seroconversion during pregnancy. Kaplan–Meier curves with log-rank tests and proportional hazards models were fitted to study seroconversion after delivery. Disturbance of vaginal flora was categorized on a scale of 0–3 as follows: 0, normal flora (reference group); 1, one criterion; 2, two criteria; and 3, BV (three or more criteria). In the regression models, disturbance of vaginal flora was included both as categorical and linear (0–3) variables. We report the results of the linear covariate in the prepartum analysis since it increased the power to detect trend, and the likelihood ratio tests indicated the data to be consistent with a continuous linear effect of disturbance of vaginal flora. In addition, due to the fact that only one of 160 pregnant women with normal flora (the reference group) seroconverted, the sparsity of data limited the reliability of categorical odds ratios (OR). In the prepartum models where disturbance of vaginal flora is thus considered linear, we show only a test for trend instead of 95% confidence intervals (CI), since the CI would have automatically not included one for each of the categories of the variable that was kept linear if the test for trend was significant. In the postpartum models, disturbance of vaginal flora is treated as a categorical variable and the score test is used for overall statistical testing.

Risk factors for seroconversion analysed in all the models included STD (gonorrhea, trichomoniasis, candidiasis, and syphilis), age (in single years), number of sexual partners (1 or ≥ 2 at baseline), time from screening to delivery (only for antenatal seroconversion analysis) and a socioeconomic status indicator (availability of electricity in the house). These variables were selected on the basis of their biological or epidemiological importance. Variables that did not contribute additional information in multivariate models were initially removed by a backward stepwise elimination procedure [12] using SAS default criteria for removal to obtain the final models (SAS Technical Report P-229, Release 6.07; SAS Institute Inc., Cary, North Carolina, USA). However, we did not entirely rely on statistical reasoning to select variables for a final model. We examined the removal of different variables from these models to assess if they made a substantial modification to the β coefficient for disturbance of vaginal flora. We removed a variable from the final model if it was not statistically significant and did not substantially (by more than 10% variation) affect the β coefficient of disturbance of vaginal flora. Where necessary, some variables were either re-entered or removed on the basis of biological and/or epidemiological evidence and the direction and magnitude of the associations were re-evaluated.

Laboratory variables that changed over time (BV and STD) were modelled as time-dependent covariates in the postpartum analysis. Women were censored at the earliest of 8 months after their last BV laboratory results or the last HIV-negative test. The choice of 8 months was based on visit intervals of 6 months and an assumed time from infection to seroconversion of about 2 months. In addition, although the follow-up clinic visits were scheduled at exactly 6 months intervals, we allowed about 2 months for a client to actually attend a specific visit. Beyond 8 months, important changes in time-dependent STDs and BV were likely to have occurred, thus making estimates of the time-dependent laboratory covariates unreliable.

HIV incidence was estimated as the number of seroconversions and is expressed per 100 person-time of follow-up. The duration of exposure to HIV was defined for antenatal women as the time from screening to delivery, and for postnatal women, from delivery to the last negative HIV test for women who remained seronegative, or the mid-point between the last negative test and the first positive test, for women who seroconverted. Calculation of the 95% CI for rates of seroconversion according to person-time was based on the Poisson distribution. Postnatal rates of BV and STDs were calculated as number of clinical conditions per 100 person-visits. These rates reflect visit prevalence and are not true incidences since a woman could have had more than one attack of the specific condition between visits. The 95% CI were calculated based on weighted means of the 6-monthly visits, adjusting for the partial collinearity of varying numbers of repeated observations from the same individuals [13]. An estimate of the population attributable risk [12] was obtained for BV alone and for all vaginal disturbance using the adjusted rate ratio (or odds ratios) and the postpartum frequency of vaginal disturbances.

Results

Prevalence of HIV and BV

Both HIV and BV were common among pregnant women in Malawi. The prevalence of HIV rose from 22% (1528 out of 6890) in 1990 to 30% (752 out of 2518) in 1993. Of 9126 women who had a pelvic examination in 1990 and 1993, 30% had BV (three or more clinical criteria), 59% (38% with one clinical criteria and 21% with two clinical criteria) had a disturbance of vaginal flora other than BV and 11% had normal vaginal flora (i.e., had none of the clinical criteria). Clue cells, the most sensitive and specific of all the clinical criteria to diagnose BV [14,15], was detected in 29% of the women examined. There were no statistically significant differences in the prevalence of BV on the basis of combined clinical criteria or presence of clue cells alone.

Disturbance of vaginal flora and HIV seroconversion during pregnancy

Of the pregnant women screened for HIV in 1990 and 1993, 1196 seronegative women were enrolled in the follow-up study. At screening during the first antenatal visit, 75% of these women were in late second trimester (approximately 6 months pregnant; median time from screening to delivery was 3.4 months). These 1196 women were followed during pregnancy for a total of 337.9 person-years (median duration of 3.4 months per woman). During this period, 27 women seroconverted; an HIV incidence of 7.99 per 100 person-years (95% CI, 4.98–11.00). The cumulative incidence of HIV at delivery was 0.6% in women with normal vaginal flora at screening, 1.8% in women with one criterion, 3.1% in women with two criteria, and 3.6% in women with BV (three or more criteria) (χ^2 trend = 5.13; $P = 0.02$).

Table 1 shows the number and rate of HIV seroconversion stratified by risk factor categories, and univariate and multivariate logistic regression results for the association of HIV seroconversion with these risk factors. In the multivariate adjusted model, compared with women with normal vaginal flora, the risk of HIV seroconversion was 1.54 among pregnant women with one criterion; 2.37 among women with two criteria; and 3.68 among women with BV. The estimated population attributable risk per cent for overall vaginal disturbances (including BV) was 48.2% and for BV alone was 22.8%.

Women with gonorrhea during pregnancy were 4.34 times more likely to seroconvert than women with no gonorrhea (Table 1). Likewise, women who were followed for a longer period from screening to delivery were more likely to seroconvert; each additional month of follow-up increased the relative risk of seroconversion 1.45 times. Although syphilis was highly associated with HIV seroconversion in the univariate model (unadjusted OR = 3.65), it was not significant ($P = 0.41$) in the multivariate model. Syphilis was removed from the final multivariate model because the change in the β coefficient of disturbance of vaginal flora with syphilis in the logistic regression model was

Table 1. Risk factors associated with HIV seroconversion among pregnant women.

Risk factors		n*	HIV seroconverters	Adjusted rate/100[†]	Unadjusted[‡] odds ratio (95% CI)	Adjusted[§] odds ratio (95% CI)
Disturbance of vaginal flora						
None		160	1	0.19	1.00[‖]	1.00[‖]
One criterion		504	9	0.53	1.55	1.54
Two criteria		221	7	0.95	2.40	2.37
Bacterial vaginosis		274	10	1.05	3.72	3.68
Linear trend					$P = 0.03$	$P = 0.04$
Gonorrhea	Yes	34	3	2.62	4.78 (1.36–16.81)	4.34 (1.20–15.70)
	No	1086	22	0.60		
Trichomoniasis	Yes	275	9	0.97	1.68 (0.74–3.82)	
	No	861	17	0.58		
Syphilis	Yes	56	4	1.87	3.65 (1.22–10.93)	
	No	1109	23	0.62		
Yeast infection	Yes	189	4	0.60	0.90 (0.31–2.65)	
	No	947	22	0.69		
Age	≥ 25 (median)	539	14	0.80	1.00 (0.94–1.06)[¶]	
	<25	626	13	0.59		
Electricity**	Yes	244	3	0.38	2.13 (0.64–7.13)	
	No	915	24	0.76		
Sexual partners	≥ Two	98	4	1.12	1.95 (0.66–5.77)	
	One	1062	23	0.64		
Time to delivery	≥ 3.4(Median)	561	15	0.59[††]	1.39 (1.25–1.83)[‡‡]	1.45 (1.08–1.95)[‡‡]
	< 3.4	604	12	0.85		

*Women missing laboratory data are excluded. [†]Adjusted for time of follow-up; rate obtained by dividing number of HIV seroconverters by person-month of follow-up for each category (person-month of follow-up not shown). [‡]Logistic regression analysis. [§]Variables included in the final stepwise logistic regression model. [‖]As a linear covariate (thus 95% CI are not given: see Methods). [¶]Age in 1 year increase in logistic regression analysis. **Index of high socioeconomic status. [††]Rate is lower because of longer antenatal follow-up period. [‡‡]Time from screening to delivery in single months. CI, Confidence interval.

only 4.9% ($\beta = 0.433$ without syphilis and $\beta = 0.412$ with syphilis in the model).

Disturbance of vaginal flora and postpartum HIV seroconversion

In the postnatal period, 1169 women were followed for a total of 2684 person-years (median duration of 2.5 years per person). During this period, 97 women seroconverted, an overall seroincidence of 3.61 per 100 person-years (95% CI, 2.89–4.33). After stratification by year since delivery, postnatal seroincidence was 2.16% during the first year, 3.02% during the second year, 5.76% during the third year, and 5.43% after the third year.

Although there were 97 seroconversions during this period, 35 of these women were censored before the date of seroconversion as more than 8 months had elapsed since their last BV (or STD) measure and first HIV-positive test. Thus, knowledge of their time-dependent BV (or STD) status near the time of seroconversion was considered unreliable. Therefore, the postnatal analysis was restricted to the 62 seroconverters with reliable time-dependent data.

Vaginal infections were common among Malawian postpartum women (Table 2). Bacterial vaginosis, trichomoniasis and candidiasis each accounted for rates of approximately 14 to 16 per 100 person-visits. The unadjusted rate ratio (RR) for postpartum HIV seroconversion was 1.54 higher among women with one criterion of vaginal disturbance compared to those with normal vaginal flora (Table 3). The relative risk of seroconversion was even higher among women with two criteria (RR = 1.71) and highest for women with BV (RR = 3.50). The overall significance of disturbance of vaginal flora by the score test was $P = 0.03$. In a multivariate proportional hazards model which included disturbance of vaginal flora as a categorical variable (three dummy variables), age, socioeconomic status, gonorrhea and trichomoniasis, only young age and having two criteria of disturbance of vaginal flora were significantly associated with HIV seroconversion. After adjusting for the above potential confounders, compared with women with normal vaginal flora, the RR for women with one criteria was 1.51 (95% CI, 0.61–3.72), for women with two criteria the RR was 3.01 (95% CI, 1.19–7.64), and for women with BV the RR was 1.84 (95% CI, 0.67–5.09) (Table 3). The overall significance of disturbance of vaginal flora by the score test was $P = 0.04$.

Although we reported in Table 3 the results of using disturbance of vaginal flora as a categorical variable, for completeness, we also report the results of using disturbance of vaginal flora as a continuous variable since it increased the power to detect trend (see Methods). In the univariate analysis, with level of vaginal disturbance fit as a linear variable, compared with women with normal flora, the RR for women with only one criterion of vaginal disturbance was 1.31, for women with two criteria the RR was 1.72, and for women with BV the RR was 2.25 (trend test $P = 0.04$). After adjusting for the same potential confounders included in Table 3 (still fitting disturbance of vaginal flora as a linear variable), compared with women with normal vaginal

Table 2. Frequency of bacterial vaginosis and sexually transmitted diseases among postpartum women.

Disease	Person-visits	Event-visits	Event rate per 100 person-visits	95% CI
Bacterial vaginosis*	4479	736	16.43	15.20–17.58
Gonorrhea	4130	44	1.07	0.75–1.39
Trichomoniasis	4015	567	14.12	12.70–15.50
Syphilis	4905	128	2.61	1.82–3.40
Candidiasis	4012	561	13.98	12.78–15.14

*Three or more clinical criteria.

Table 3. Association of risk factors with HIV seroconversion among postpartum women*.

Risk factors	Unadjusted risk ratio (95% CI)	Adjusted† risk ratio (95% CI)
Disturbance of vaginal flora		
None	1.00‡	1.00§
One criterion	1.54 (0.81–2.93)	1.51 (0.61–3.72)
Two criteria	1.71 (0.86–3.38)	3.01 (1.19–7.64)
Bacterial vaginosis‖	3.50 (1.47–8.31)	1.84 (0.67–5.09)
Gonorrhea present	3.11 (0.76–12.79)	2.96 (0.71–12.29)
Trichomoniasis detected	1.88 (1.32–3.38)	1.38 (0.75–2.56)
Syphilis reactive	1.04 (0.24–4.28)	
Yeast infection	0.84 (0.40–1.76)	
Age (1 year increase)	0.91 (0.87–0.95)	0.91 (0.87–0.96)
Electricity in the house¶	2.19 (1.00–4.80)	1.98 (0.90–4.36)
Sexual partners ≥ 2	1.28 (0.58–2.82)	

*Proportional hazards analysis. †Variables included in the final multivariate proportional hazards regression model. ‡χ^2 (3 degrees of freedom) = 8.82, $P = 0.03$. §χ^2(3 degrees of freedom) = 8.06, $P = 0.04$. ‖Three or more criteria. ¶Index of high socioeconomic status.

flora, the RR for HIV seroconversion among women with one criterion of disturbance of vaginal flora was 1.32, for women with two criteria was 1.74, and for women with BV was 2.30 (trend test $P = 0.03$). Similar to the model where disturbance of vaginal flora was defined as a categorical variable (Table 3), in the multivariate models with disturbance of vaginal flora fitted as a linear variable, young maternal age (RR = 0.91, 95% CI, 0.87–0.95) was the only additional variable which was statistically significant. Neither gonorrhea, trichomoniasis or socioeconomic status were significantly associated with postpartum HIV seroconversion in these models. The estimated population attributable risk per cent of HIV seroconversion for overall vaginal disturbances (including BV) was 33.0% and for BV alone was 14.3%.

Discussion

This study suggests that disturbance of vaginal flora is significantly associated with increased risk of HIV acquisition in both pregnant and postnatal women. During pregnancy seroconversion risk was highest in women with BV (adjusted OR = 3.7), intermediate in women with two clinical criteria (adjusted OR = 2.4) and lowest in women with only one criterion (adjusted OR = 1.5) ($P = 0.03$ linear tend). Among postpartum women there was a significant association between HIV seroconversion and intermediate disturbance of vaginal flora (two clinical criteria in this study). The lack of a significant association between BV (three or more clinical criteria) and HIV seroconversion in the postpartum analysis, when disturbance of vaginal flora was entered as three distinct dummy variables (categorical) in the multivariate model, (Table 3) could be due to a type II error. The upper limit of the 95% CI for the RR of BV was 5.09 and the overall *P*-value for this categorical variable was 0.04. In the multivariate model where disturbance of vaginal flora was defined as a continuous variable, there was a significant trend ($P = 0.03$) of association between increasing disturbance of vaginal flora and postpartum HIV seroconversion.

This investigation confirms the findings of earlier cross-sectional studies which reported associations between BV and HIV among commercial sex workers in Thailand [16], rural women in the Rakai district of Uganda [1] and urban women in Malawi [17]. The temporal nature of the association in this study strengthens the inference that the relationship between BV and HIV acquisition could be causal.

The current data and previous reports from Malawi [18], show that STDs are common and represent a major problem among pregnant and postpartum women. For example, gonorrhea, syphilis and trichomoniasis were significantly associated with HIV seroconversion either in univariate or multivariate models. Gonorrhea and syphilis showed large relative risks, although their incidence and prevalence rates were not as high as those of BV or trichomoniasis.

Other risk factors significantly associated with higher HIV seroconversion were longer time from screening to delivery and younger maternal age (Tables 1 and 3). Neither of these is surprising as younger age is commonly associated with greater infection [18], and a longer time between entry and delivery should increase the probability to seroconvert given that seroconversion is a function of time. The length of time from screening to delivery is probably a correlate for sexual activity since sexual abstinence is common in late pregnancy and in the early postnatal period among Malawian women [19]. Decreased sexual activity during late pregnancy and following delivery could explain the lower HIV incidence during the first year after delivery (2.16 per 100 person-years; the lowest rate in our postnatal follow-up).

Risk factors not measured in this investigation, could be a source for potential confounding. We did not test for HIV or collect data on the male partners of the women in this study, and therefore, some risk factors might not have been adequately addressed. For example, we have no data to determine if a male HIV-infected partner had gonorrhea or trichomoniasis, and if such STDs have been transmitted to the female partner and have confounded the association between BV and HIV seroconversion. We have controlled, however, for sexual activity (number of sexual partners) and STDs in our analyses. Sexual transmission of BV is unlikely since several studies have shown that treatment of male partners of women with BV did not reduce the rate of recurrence of the condition in women [20,21]. Lack of longitudinal measurements of changes in sexual behavior including condom use is a possible limitation. We included baseline number of sexual partners as a surrogate for sexual activity, and this was not significantly associated with HIV seroconversion either prenatally or postnatally. As reported in earlier studies from Malawi [18,22], the rates of life-time condom use were low (6% in 1990, 18% in 1993, and 5% in 1995), and inconsistent use of condoms was common. We did not regularly perform chlamydia testing on all women under follow-up. The prevalence of chlamydia has been shown to be low among women tested in the antenatal clinic, and in a recent study among men attending a STD clinic in Malawi, a prevalence of only 2% was reported [23].

Despite the strong relationship of both BV and STDs with sexual activity, it is not clear if the presence of one facilitates the transmission of the other [6]. However,

STDs (ulcerative and non-ulcerative) are known to facilitate HIV transmission by increasing infectiousness of HIV-infected individuals or rendering HIV-uninfected individuals more susceptible to HIV infection. Potential biological mechanisms for this inter-relationship of STDs and HIV include increased shedding of the virus in genital fluids, recruitment of HIV target cells or HIV-infected cells into the genital tract as part of the inflammatory process, stimulation of immune response to an STD causing increased viral replication, and disruption of protective epithelial barriers [24–27]. Direct evidence on the association of STDs and HIV was also provided by a large randomized community-based trial in Tanzania which showed that improved treatment of STDs can lower HIV incidence by about 40% [28]. Therefore, including treatment of BV in interventions of STDs could be a valuable approach.

Several factors could lead to the acquisition of HIV when the normal vaginal microflora are disturbed. First, depletion of lactobacilli may limit production of hydrogen peroxide and other antibacterial activities which are protective against potentially pathogenic organisms such as STDs and possibly HIV [29,30]. Second, low vaginal pH has been postulated to inhibit CD4 lymphocyte activation and reduce HIV target cells in the vagina [8]. Therefore, lack of lactic acid production by lactobacilli could lead to an elevated pH which may be more conducive to growth and survival of the virus [9]. Third, similar to experience with several bacteria [31], elevated vaginal pH may enhance HIV adherence to vaginal eukaryotic cells.

It is unlikely that the clinical definition of BV we adopted in this study, compared with a diagnosis based on a Gram stain, has greatly influenced our results. The Gram stain and clinical diagnosis of BV were shown in several studies to have a high correlation [14,32]. The prevalence of BV was comparable whether we used the conventional composite clinical criteria or the presence of clue cells. Inclusion of vaginal discharge in the clinical definition without restrictions related to concurrent STDs (as described in the Methods) did not influence the prevalence of BV and had minimal effect on the prevalence of other vaginal disturbances (data not shown). The criteria we used are available as part of the routine laboratory testing for STDs and require no additional training compared to the expertise needed to make proper evaluation of a Gram stain. We opted to use a broad classification of vaginal disturbance reflecting the number of observed criteria because our data showed an increasing trend of seroconversion with increasing number of criteria. This suggests either increasing severity of vaginal disturbance or certainty of diagnosis. The finding that only 11% of women in this study had normal vaginal flora underscores the high frequency and potential importance of the clinical spectrum of disturbances of vaginal flora. For example, women with intermediate flora were reported to be more likely to acquire BV than women with normal flora [3].

Our data suggest that the risk of seroconversion was higher during pregnancy than postnatally (relative risk of 3.7 and attributable risk of 23% during pregnancy compared to a relative risk of 2.3 and attributable risk of 14% postnatally). Pregnancy associated factors such as high levels of oestrogen [5] and local cervical factors (increased vascularity, exudation and ectopy) [33], coupled with sexually transmitted infections (e.g. gonorrhea), could increase the susceptibility to HIV infection during pregnancy. Therefore, BV could substantially increase transmission of HIV from the mother to the infant. Additionally, BV has been implicated in several serious obstetric and gynaecologic sequelae such as preterm delivery, premature rupture of membranes, amniotic fluid infection, chorioamnionitis, postpartum endometritis and pelvic inflammatory disease [14,34–36]. Screening and treating pregnant women with BV could reduce several adverse reproductive outcomes including perinatal transmission of HIV. Treatment of BV could restore normal vaginal flora and reduce the susceptibility of women to HIV infection. Simple and effective treatment and prevention measures are urgently needed.

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