Incident Herpes Simplex Virus Type 2 Infection Increases the Risk of Subsequent Episodes of Bacterial Vaginosis

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Herpes simplex virus type 2 (HSV-2) infected women have a higher prevalence of bacterial vaginosis (BV) compared to HSV-2-seronegative women. To explore the temporal association between these conditions, we evaluated the frequency of BV episodes before and after HSV-2 acquisition in a prospective study of 406 HSV-2/HIV-1-seronegative Kenyan women, of whom 164 acquired HSV-2. Incident HSV-2 was associated with increased likelihood of BV (adjusted OR, 1.28; 95% CI, 1.05–1.56; *P* = .01). Our findings strengthen the evidence for a causal link between genital HSV-2 infection and disruption of the vaginal microbiota.

Keywords. Bacterial vaginosis; herpes simplex virus type 2; women; Africa.

Bacterial vaginosis (BV) is a polymicrobial condition characterized by depletion of hydrogen-peroxide producing vaginal lactobacilli and overgrowth of *Gardnerella vaginalis* and other anaerobic bacteria [1]. Although BV is the most common cause of abnormal vaginal discharge, 50%–75% of women with BV remain asymptomatic. Bacterial vaginosis is common worldwide among women of reproductive age. In the United States, the estimated prevalence of BV among women aged 14–49 is

29% [2]. Among African women, BV prevalence has been reported to be as high as 51% [3]. Bacterial vaginosis has been associated with increased risk of sexually transmitted infections (STIs) including human immunodeficiency virus type-1 (HIV-1), and with adverse reproductive health outcomes.

Herpes simplex virus type-2 (HSV-2) is a common STI worldwide and the leading cause of genital ulcer disease [4]. Most HSV-2 infections are asymptomatic, with >80% of HSV-2 seropositive individuals asymptomatically shedding virus. It is estimated that 23% of women in the United States [5] and over 50% of women in sub-Saharan Africa are infected with HSV-2 [6]. In 2003, 536 million people were infected with HSV-2 globally [4], and HSV-2 incidence was 23.6 million new cases per year. HSV-2 infection is more common in women than men. The prevalence of this chronic infection increases with age. Among high-risk groups, HSV-2 incidence can be remarkably high. For example, we reported an annual HSV-2 incidence of 23% among high-risk women in Mombasa, Kenya [7]. Like BV, HSV-2 has been found to be a significant risk factor for HIV-1 acquisition [8].

Several studies have observed associations between HSV-2 and BV. Women with BV are more likely to acquire other STIs including HSV-2 [9]. In addition, women with prevalent HSV-2 infection have a higher incidence of BV compared to HSV-2 uninfected women [10]. This observation could suggest that HSV-2 increases the risk of BV. Alternatively, women with more frequent BV may simply be more likely to acquire HSV-2. To distinguish between these 2 possibilities, we compared women's likelihood of having BV before and after HSV-2 acquisition.

MATERIALS AND METHODS

We conducted longitudinal follow-up of women participating in an open cohort study of high-risk women in Mombasa, Kenya, between February 1993 and February 2011. The eligibility criteria to join the cohort were age 18–50 years, residing in the Mombasa area, self-identifying as exchanging sex for payment in cash or in kind, and able to provide informed consent. This study was approved by the ethics review boards of Kenyatta National Hospital and the University of Washington. All participants provided written informed consent.

Clinic Procedures

At enrollment and monthly follow-up visits, a study nurse conducted a standardized face-to-face interview covering demographic data and medical and sexual history. A study physician performed a physical examination including a pelvic speculum

examination. Swabs of cervical and vaginal secretions were collected for laboratory diagnosis of STIs. Blood samples were collected for HSV-2 and HIV-1 testing. Participants were provided free outpatient medical services including treatment of STIs according to Kenyan national guidelines. If indicated, syndromic management was offered during the examination visit. Participants were asked to return for test results after 7 days. At the results visit, additional treatment was provided for infections diagnosed by laboratory testing that had not been treated with syndromic management at the prior visit.

Laboratory Procedures

Serological testing for HSV-2 was performed using a type-specific HSV-2 gG based enzyme-linked immunosorbent assay (ELISA; HerpeSelect 2, Focus Diagnostics, Cypress, CA) on archived samples. An index value of <2.1 (the ratio of the optical density [OD] of the sample to the OD of the standard calibrator) was recorded as negative. Index values greater than or equal to 2.1 were considered to be positive. We selected this cutoff as likely providing the best balance of sensitivity and specificity, based on a prior study that found 2.1 to be the optimum assay cutoff in African populations similar to our own. The study demonstrated that this cutoff had 93.9% sensitivity and 90.5% specificity, against a gold standard HSV-2 Western blot. This was in comparison to the manufacturer's cutoff of >1.1, which had a sensitivity and specificity of 98.3% and 80.3%, respectively [11]. Vaginal Gram-stained slides were scored using Nugent's criteria. Scores ≥7 were classified as BV. Vaginal saline wet preparations were assessed for the presence of motile trichomonads and yeast. Culture for *Neisseria gonorrhoeae* was performed on modified Thayer Martin media. Cervical Gram-stained slides were examined microscopically for the presence of Gram-negative intracellular diplococci. Beginning in 2006, endocervical samples were tested for the presence of *N. gonorrhoeae* and *Chlamydia trachomatis* by transcription mediated amplification (TMA) using the Gen-Probe Aptima GC/CT Detection System (Gen-Probe, San Diego, CA). HIV-1 serostatus was determined by ELISA (Detect HIV1/2, BioChem Immunosystems, Montreal, Canada, or PT-HIV 1,2–96, Pishtaz Teb Diagnostics, Tehran, Iran). Positive tests were confirmed using a second ELISA (Recombigen, Cambridge Biotech, Worcester, MA, or Vironostika HIV-1 Uniform II AG/AB, bioMerieux, Marcy l'Etoile, France).

Data Analyses

We included all HIV-1-seronegative women in the cohort who were initially HSV-2 seronegative. For women who acquired HIV-1 during the study, we censored visits following HIV-1 infection. The primary exposure of interest was incident HSV-2 infection. Women were considered HSV-2 uninfected prior to a positive HSV-2 test and positive thereafter. The outcome was BV, dichotomized according to the presence or absence of BV (Nugent score 7–10 vs 0–6). The prevalence of BV was compared during HSV-2-seronegative vs HSV-2-seropositive follow-up visits. The outcome (BV) was measured at multiple time points on participants in our study; thus we used generalized estimating equation (GEE) modeling to allow us to assess the association between incident HSV-2 and BV while accounting for the correlation induced by having multiple observations per individual participant. We used GEE with a logit link, exchangeable correlation structure, and robust variance estimates. Results were expressed as odds ratios (OR) with 95% confidence intervals (CI). We considered known and suspected potential confounding factors including age, place of work (bar/restaurant vs nightclub or home-based/other), education level, marital status, sexual risk behaviors, STIs, and other genital tract infections (*N. gonorrhoeae, C. trachomatis, Trichomonas vaginalis, Candida albicans*) hormonal contraceptive use, vaginal washing, alcohol consumption, and tobacco use. We assessed the effect of potential confounding factors one at a time on BV. In the final adjusted model, we included variables that changed the regression coefficients for HSV-2 serostatus as a predictor of BV by 10% or more. We also conducted a sensitivity analysis restricting the data to the subset of women who acquired HSV-2. Analyses were performed using PASW 18.0 (PASW Inc., Chicago, IL) and STATA 11 (StataCorp, College Station, TX).

RESULTS

Between February 1993 and February 2011, 406 women who were both HIV-1 and HSV-2 seronegative contributed 809 person-years of follow-up at 5650 visits. The median duration of follow-up was 652 days (interquartile range [IQR], 147–1852). The median age of the participants at baseline was 24 years (IQR, 22–28). Most women worked in bars (N = 244, 60%), whereas the remainder worked in nightclubs (N = 145, 36%) or at other venues (N = 17, 4%). One hundred and nineteen women (29%) reported unprotected intercourse at baseline. Bacterial vaginosis was present at baseline in 116 (29%) of participants. Ninety-two percent of the women reported that they performed vaginal washing.

There were 164 incident HSV-2 infections (incidence rate 21/100 person-years). The prevalence of BV was higher during visits after HSV-2 seroconversion compared to visits before HSV-2 seroconversion (Figure 1). After adjustment for age, incident HSV-2 infection was associated with 1.28-fold increase in the odds of BV (95% CI, 1.05–1.56; *P* = .01) (Table 1). The magnitude of this association was similar in sensitivity analyses limited to the 164 women who acquired HSV-2 (adjusted OR, 1.25; 95% CI, 1.00–1.57; *P* = .05).

DISCUSSION

In this cohort of HIV-1-seronegative women, we found that incident HSV-2 infection was associated with an approximately
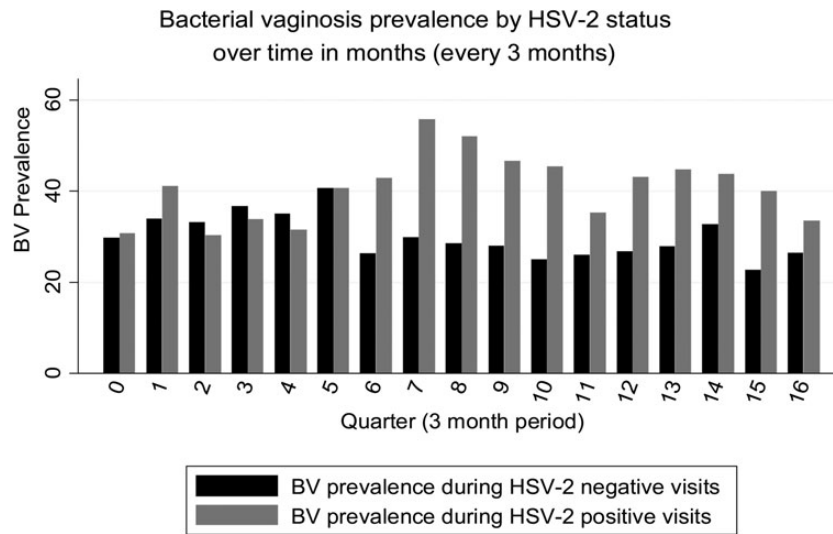
Figure 1. Time in months since enrollment into the cohort. All women were HSV-2 negative at baseline. As the number of months in follow-up increases, there is an increase in the proportion of women with HSV-2. The proportion of women in follow-up who were HSV-2 positive at 6, 12, 18, 24, 30, 36, 42, and 48 months is 19%, 23%, 23%, 29%, 33%, 38%, 47%, and 66%, respectively. The prevalence of BV in HSV-2 negative and HSV-2 positive women is shown for every 3 months. Data are collapsed after month 48 due to sparse data after 4 years. Abbreviations: BV, bacterial vaginosis; HSV-2, herpes simplex virus type 2.

30% increase in the odds of episodes of BV. These findings advance our understanding of the association between HSV-2 infection and the vaginal microbiota, highlighting the temporal relationship between incident HSV-2 infection and a subsequent increase in the frequency of BV. By characterizing the temporal relationship between HSV-2 acquisition and increased episodes of BV, this study makes a valuable contribution that extends beyond earlier prospective studies [10, 12]. The magnitude of the association between HSV-2 infection and increased risk of BV that was observed in this study was relatively similar to that observed in prior studies relating prevalent HSV-2 infection to BV [10, 12].

The biological mechanisms that might be responsible for increases in BV following HSV-2 infection are not clear. One possible mechanism is that intermittent HSV-2 reactivation may lead to immune activation in the genital mucosa, altering the vaginal microbiota [13]. Another plausible biological mechanism is that *G. vaginalis* depends on having a source of iron to thrive [14]. This may be particularly important between menses, when availability of iron could be a limiting factor. More consistent availability of iron may create an environment that facilitates the growth of *G. vaginalis*. Additional studies will be required to elucidate the biological link between HSV-2 infection and BV.

Table 1. Prevalence of BV During HSV-2 Negative vs HSV-2 Positive Follow-up

	HSV-2 Negative Follow-up Visits	HSV-2 Positive Follow-up Visits	OR (95% CI)	P Value*	aOR** (95% CI)	P Value*
	N = 3769	N = 1881				
BV prevalence, all women[a]	1173 (31.1%)	689 (36.6%)	1.19 (.99–1.44)	.07	1.28 (1.05–1.56)	.01
	N = 1296	N = 1881				
BV prevalence, HSV-2 seroconverting women[b]	424 (32.7%)	689 (36.6%)	1.12 (.91–1.36)	.29	1.25 (1.00–1.57)	.05

Abbreviations: aOR, adjusted odds ratio; BV, bacterial vaginosis; CI, confidence interval; HSV-2, herpes simplex virus type 2; OR, odds ratio.

[a] Nugent score 7–10 (vs 0–6).

[b] Nugent score 7–10 (vs 0–6), limited to the 164 women who acquired HSV-2.

*P-values generated from models using generalized estimating equations with a logit link, exchangeable correlation structure and robust errors.

**Model adjusted for age. Additional covariates considered for the multivariate model included place of work, education level, marital status, sexual risk behaviors, sexually transmitted infections, hormonal contraceptive use, vaginal washing, alcohol consumption, and tobacco use. However, these covariates did not confound the association between incident HSV-2 infection and BV prevalence, so were not retained in the final model.

It is interesting to note that the increased likelihood of BV following HSV-2 infection could serve as a mechanism for enhancing further herpes transmission because BV increases genital shedding of HSV-2 [9, 12, 15]. In addition, both HSV-2 and BV have been associated with a greater risk of acquiring and transmitting HIV-1 [8]. Thus, understanding the synergistic interactions between BV and HSV-2 could have important HIV-1 prevention implications. Immunodeficiency caused by HIV-1 infection also increases the frequency and severity of HSV-2 reactivations, which could result in increased BV episodes in HIV-1-positive women. Thus, HIV-1 status is an important consideration when assessing the association between BV and HSV-2 infection.

Our study had several strengths. First, these data were prospectively collected from a large population, allowing us to accrue a substantial number of incident cases of HSV-2 infection. The large sample and prolonged follow-up provided statistical power, which allowed us to establish the temporal relationship between HSV-2 infection and increased detection of BV. Second, we had a relatively homogenous population, such that women who acquired HSV-2 were similar to those who did not. Moreover, our analyses provided similar results even when we restricted only to those women who acquired HSV-2. Third, frequent cohort visits allowed us to identify the timing of HSV-2 infection with a high level of precision.

Our results should be interpreted in the context of a number of limitations. First, this was an observational study. Thus, it is not possible to definitively prove that HSV-2 infection caused an increase in BV episodes. Second, of the 406 participants in the study, 35 (8.6%) had an initial index value between 1.1 (manufacturer's recommended cutoff) and 2.1, and then progressed to an index value >2.1. Unfortunately, we do not have Western blot data for these samples. Thus, it is possible that the cutoff of 2.1 resulted in some participants with index values between 1.1 and 2.1 being falsely classified as negative. Third, we did not collect monthly specimens for HSV-2 detection. This would have served to strengthen our argument that increases in BV may result from intermittent HSV-2 reactivation. Future studies assessing the association between HSV-2 and vaginal microbiota should consider measuring HSV-2 shedding at the time of BV assessment, and more frequently if feasible. Finally, our study population was composed of high-risk women who reported exchanging sex for payment in cash or in kind. These women's sexual risk behavior is expected to be different from the general population, and this could limit the generalizability of our findings.

By demonstrating the temporal sequence of HSV-2 infection followed by an increase in the likelihood of BV, these results strengthen the evidence for a causal link between genital herpes infection and disruption of the vaginal microbiota. Additional studies are needed to improve our understanding of the biological basis of increased BV prevalence among women who become infected with HSV-2. It will also be important to determine whether prevention or suppression of HSV-2 infection is associated with less frequent episodes of BV.

Notes

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