HSV

Association between bacterial vaginosis and *Herpes simplex* virus type-2 infection: implications for HIV acquisition studies

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Objectives: Bacterial vaginosis (BV) and *Herpes simplex* virus type-2 (HSV-2) have been linked to an increased risk of HIV-1 acquisition. Recent research suggests an association between BV and HSV-2 acquisition, but the converse has not been studied. Here, we investigate whether an association exists between BV and HSV-2 infection

Methods: We examined the determinants of BV occurrence in a cohort of female sex workers in Burkina Faso. Participants were followed every 3 months for diagnosis of genital infections and report of sexual behaviours. Factors associated with BV occurrence were assessed using generalised estimating equation models.

Results: We enrolled 273 women (mean age, 28 years) and conducted 812 follow-up visits (mean 2.93 visit per woman). Baseline seroprevalence of HIV-1, HSV-2 and recent syphilis were 31.5%, 70.1% and 0.4%, respectively, while baseline prevalence of BV, *Trichomonas vaginalis* (TV) and *Candida albicans* were 20.5%, 3.3% and 2.5%, respectively. In multivariable analysis, HSV-2 (relative risk (RR) = 1.73, 95% CI 1.12 to 2.65), HIV-1 (RR = 1.76, 95% CI 1.30 to 2.40), TV (RR = 1.5, 95% CI 1.0 to 2.3), and having ⩾3 sexual partners in the preceding week (RR = 2.2, 95% CI 1.1 to 4.6) were independently associated with BV, while hormonal contraception showed a protective effect (RR = 0.11, 95% CI 0.02 to 0.70).

Conclusions: HSV-2 infection was associated with BV occurrence in this population. As HSV-2 is strongly linked to HIV-1 acquisition, studies assessing the cofactor effect of BV on HIV acquisition should control for the presence of HSV-2. Further studies are required to investigate the relative effect of asymptomatic HSV-2 shedding and/or genital ulcerations on BV occurrence.

Bacterial vaginosis (BV) is a polymicrobial syndrome that represents the main cause of abnormal vaginal discharge worldwide, with prevalence in the general population ranging from 20 to 50%.[1] Several studies have suggested bilateral interactions between BV and HIV-1. Cross-sectional studies have reported that HIV-seropositive women were more likely to have BV episodes.[2] Reciprocally, longitudinal studies have suggested that BV could facilitate HIV acquisition.[3–5]

One of the strongest epidemiological correlates of HIV acquisition is infection with *Herpes simplex* virus type-2 (HSV-2),[6] through clinical episodes and asymptomatic genital shedding. Recent research suggests that BV could facilitate HSV-2 acquisition[7] and also trigger HSV-2 genital shedding episodes.[8] However, there is little available information regarding the potential role of HSV-2 infection upon the occurrence of BV episodes. We examined the determinants of BV occurrence in a cohort of high-risk women in Burkina Faso, West Africa, with a particular focus on the hitherto little-studied role of HSV-2 infection.

METHODS

This study was conducted within a cohort study of female sex workers (FSWs) in Bobo-Dioulasso, Burkina Faso, which has been described previously.[9] In brief, FSWs who provided written informed consent were invited to join an intervention and research programme on HIV and other sexually transmitted infections (STI). At enrolment and subsequent 3-month follow-up visits, participants provided socio-demographic and behavioural information through face-to-face interviews and underwent clinical examination, during which cervical and vaginal swabs for the diagnosis of genital infections were collected. Genital ulcer aetiology was not determined in this study. Blood samples were collected at baseline for HIV, HSV-2 and syphilis serologies.

HIV and STI prevention education sessions, as well as condom promotion and distribution, were provided at each visit. Consultation with a psychologist for either HIV pre-test counselling or support was also provided. All medical care, including investigations and treatment, were provided for free. Free provision of highly active antiretroviral therapy (HAART) was only initiated in early 2004 in this cohort. STIs were treated at each visit according to World Health Organization (WHO) recommended and locally-adapted guidelines, following the syndromic approach. Women with abnormal vaginal discharge syndrome were given a single dose of 2 g metronidazole to cover for BV and *Trichomonas vaginalis* (TV), in addition to a 10-day Nystatin regimen for genital candidiasis. At the time of study, algorithms for genital ulcer disease did not include treatment for genital herpes.

The study was approved by the Centre Muraz institutional ethical committee and the research ethics committee of the Ministry of Health of Burkina Faso.

Laboratory analyses

Presence of HSV-2 serum antibodies was detected using a specific IgG2 ELISA test (Kalon Biologicals Ltd, Aldershot, UK), shown to have high sensitivity (92.3%) and specificity (97.7%) in African samples.[10] HIV-1 antibodies were detected using a well-validated strategy based on two complementary ELISAs.[11] Recent syphilis was defined as dual seroreactivity to rapid plasma reagin test (RPR, Human GmbH, Wiesbaden, Germany) and *Treponema pallidum* haemagglutination assay (TPHA, Newmarket Laboratories Ltd, Kentford, UK). Other laboratory tests included immediate direct microscopy for the diagnosis of TV and cultures on specific media for *Neisseria gonorrhoeae* (NG) and *Candida albicans* (CA). BV was diagnosed on Gram-stained vaginal smears using the Nugent scoring method (score⩾7).

Chlamydia trachomatis was not tested for in this study, but prevalence as determined by PCR in this population had been shown to be low (2%).[9] An internal quality assurance scheme was organised for all laboratory tests by an experienced biologist. Agreement for Nugent's scoring categories was 95% (data not shown), and final diagnostic decision in case of discrepancy was based on the experienced microbiologist's results.

Statistical analyses

We describe the pattern of BV occurrence in this cohort using per-participant summary measures. Overall incidence was defined by the total number of BV episodes divided by the duration of follow-up expressed in person-years (py).

In order to identify the determinants of BV occurrence, we used a generalised estimating equation (GEE) model that allows for repeated measurements in the same individual. This model was based on a log link function with an exchangeable covariance structure for the error terms. Factors with unadjusted relative risks (RR) $\geqslant$1.5 or $\leqslant$0.5 by univariable analysis were selected for multivariable analysis. Unadjusted and adjusted RR, 95% CI and p values using the Wald test are reported. These analyses were decided upon after completion of the study.

RESULTS

Between February 2002 and August 2003, a total of 279 women were followed at the study clinic and 273 were included in this analysis (6 women were excluded because of missing BV data). A total of 812 follow-up visits were performed with a mean of 2.97 visits per woman (range 1–6) over a mean period of 8.5 months.

Study participants had a mean age of 28 years (range 16–54). At baseline, 100% condom use with first-time clients was reported by 84.3% of women. Hormonal contraceptives were only used by 4.0% of women. The majority of participants reported practising daily vaginal douching (259/268, 96.6%), using only soap and/or water for the majority (93.4%). Seroprevalence of HIV-1, HSV-2 and recent syphilis were 31.5%, 70.1% and 0.4%, respectively. Baseline prevalence of BV, TV and CA were 20.5%, 3.3% and 2.5% respectively, while no cases of NG were recorded.

Occurrence of at least one BV episode was experienced by 119 (43.6%) women, of whom 64.7%, 26.1% and 9.2% experienced one, two or three or more episodes, respectively. Overall BV incidence was 1.03 episode/py.

HSV-2, HIV-1, TV, vaginal douching and the report of three sexual partners or more in the week preceding the follow-up visit, were factors associated with a higher risk (RR$>$1.5) of BV occurrence in univariable analysis, whilst hormonal contraception (including oral and injectable hormones) showed a protective effect (RR$<$0.5) (table 1). In multivariable analysis based on 668 visits (after exclusion of visits with missing data), the same factors, with the exception of vaginal douching, remained significantly and independently associated with BV occurrence: HSV-2 (RR = 1.73, p = 0.01), HIV-1 (RR = 1.76, p$<$0.001), TV (RR = 1.5, p = 0.04) and having $\geqslant$3 recent sexual partners (RR = 2.2, p = 0.03) were associated with increased risk, whilst hormonal contraception (RR = 0.11, p = 0.02) was associated with a lowered risk. There was no interaction between HIV-1 and HSV-2 with respect to the outcome as assessed by inserting an interaction term between the two variables in the final model (p = 0.81).

DISCUSSION

We found that HSV-2 seropositive women, whether HIV-seropositive or not, were at greater risk of experiencing BV compared to HSV-2 seronegative women. To our knowledge, this association between HSV-2 serostatus and BV occurrence has never been previously reported. Possible explanations are that intermittent HSV-2 genital shedding in HSV-2 seropositive women could disrupt vaginal flora, be linked to hormonal changes, or both, which could in turn trigger BV episodes. In support of the former hypothesis, a longitudinal American study assessing the determinants of genital HSV-2 shedding among HSV-2 seropositive women found that BV (altered vaginal flora) was strongly associated with HSV-2 shedding episodes (OR = 2.3, 95% CI 1.3 to 4.0).[8] In support of the latter, the consistent protective effect of hormonal contraceptive methods on BV occurrence[12] and the cyclical occurrence of BV following menstruation, as suggested by a longitudinal study of BV incidence in The Gambia,[13] point to a hormonal influence in the occurrence of BV. HSV reactivations could result from a variety of poorly understood stimuli including changes of vaginal pH or menstruation,[14] which suggests at least an indirect hormonal influence in triggering HSV and BV episodes. This hypothesis is supported by the association between hormonal contraceptives and HSV-2 shedding found in the longitudinal American study mentioned above.[8] Clearly, further studies are required to investigate the epidemiological and biological relationships between BV and HSV-2 infection. Importantly, it would be useful to investigate the relative effect of asymptomatic HSV-2 shedding and/or genital ulcerations on BV occurrence. Unfortunately this could not be performed in this study.

Our data are in accord with previous findings regarding other risk factors for BV. HIV infection has been shown to be a major risk for BV development.[2] STIs such as TV and some sexual behaviour indicators have also been associated with BV occurrence,[15] although the mechanisms underlying their effect on BV are poorly understood, unless all are markers of a concurrent STI, such as HSV-2. Vaginal douching has been cited as a possible aetiological factor, but a large study in The Gambia[16] did not find any association between vaginal hygiene practices and BV. Similarly, our population reported high levels of vaginal douching but this was not linked to BV occurrence in multivariable analysis. As reported previously, the prevalence of parasitic or bacterial STIs was low in our study population.[17]

Our study had some limitations. We might have missed some BV episodes that could have occurred between follow-up visits. Moreover, our results are based on a high-risk population with specific sexual behaviours, and might not be applicable to general female populations in Africa. However, even if BV incidence was different in general populations, we believe that the likely biological mechanisms underlying the association between HSV-2 and BV would remain.

When considering together our findings supporting the hypothesis of an association between HSV-2 and BV and the strong epidemiological link between HSV-2 and HIV acquisition, it becomes plausible that previous studies reporting an increased risk of acquiring HIV-1 in women with BV might have been biased by confounding. Indeed, none of the three longitudinal studies[3–5] that have reported these associations controlled for the presence of HSV-2 infection. Conversely, it is noteworthy that the large community-based STI intervention trial performed in Rakai, Uganda, failed to show an association between abnormal bacterial vaginosis and HIV-1 acquisition,[18] despite an initial suggestion of strong associations based on their baseline cross-sectional study.[2] In addition, two trials of presumptive antibiotic prophylaxis to reduce HIV-1 acquisition showed a marked decrease of BV occurrence without any impact on HIV incidence.[19 20] The lack of an association between BV and HIV-1 incidence was also noted in Burkina Faso, although that study might have had limited statistical power.[9]

In summary, our findings highlight the need to further investigate the relationships between BV, HSV-2 and HIV, at

Table 1 Factors associated with BV occurrence among 273 high-risk women in Bobo-Dioulasso, Burkina Faso, in uni- and multivariable analyses

Factors	No. of visits	No. of visits with BV (%)	Unadjusted RR (95% CI)	Adjusted RR (95% CI)	p Value* for adjusted RR
Age groups (years):					
16–24	358	74 (23.0%)	1.0		
25–34	251	55 (25.1%)	1.1 (0.8–1.6)		
35–54	201	45 (24.9%)	1.1 (0.7–1.6)		
Married:					
No	699	158 (25.3%)	1.0		
Yes	111	18 (18.4%)	0.8 (0.4–1.3)		
Always use condoms:					
No	425	94 (25.1%)	1.0		
Yes	250	55 (23.9%)	1.0 (0.7–1.4)		
Number of sexual partners in preceding week:					
0	51	6 (13.3%)	1.0	1.0	
1–2	247	51 (22.3%)	1.7 (0.9–3.4)	2.0 (0.9–4.2)	0.08
⩾3	476	111 (26.6%)	2.0 (1.0–3.9)	2.2 (1.1–4.6)	0.03
Vaginal douching:					
No	44	6 (14.6%)	1.0	1.0	
Yes	757	169 (25.1%)	1.5 (0.8–3.1)	1.4 (0.8–2.7)	0.28
Frequency of douching each day:					
0	44	6 (14.6%)	1.0		
1–2	568	123 (23.6%)	1.5 (0.7–2.9)		
⩾3	137	35 (30.2%)	1.8 (0.9–3.6)		
Hormonal contraception:					
No	777	175 (25.3%)	1.0	1.0	
Yes	35	1 (3.1%)	0.1 (0.0–0.7)	0.1 (0.0–0.7)	0.02
Used antibiotics last week:					
No	643	137 (23.7%)	1.0		
Yes	129	31 (27.4%)	1.1 (0.7–1.6)		
Candida albicans:					
Absent	683	167 (24.5%)	1.0		
Present	41	9 (22.0%)	0.9 (0.6–1.5)		
Trichomonas vaginalis:					
Absent	703	167 (23.8%)	1.0	1.0	
Present	21	9 (42.9%)	1.7 (1.0–2.8)	1.5 (1.0–2.3)	0.04
HSV-2 serology:					
Negative	233	29 (13.6%)	1.0	1.0	
Positive	536	139 (29.2%)	2.1 (1.4–3.2)	1.7 (1.1–2.7)	0.01
HIV-1 serology:					
Negative	568	93 (18.5%)	1.0	1.0	
Positive	244	83 (37.6%)	2.0 (1.5–2.7)	1.8 (1.3–2.4)	<0.001

RR, relative risk.
*based on Wald test. Adjustment was made for all variables with a result shown in the adjusted RR column.

the epidemiological level, using appropriate statistical adjustments, and at the biological level to further elucidate the mechanisms of their interactions. This would have important implications for the proper interpretation of studies analysing cofactors of HIV acquisition, including BV.

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Key messages

- HSV-2 infection is associated with occurrence of BV episodes, and this association persists whatever the HIV status
- The role of HSV-2 asymptomatic genital shedding and/or genital ulcerations in the development of BV episodes should be further investigated
- Studies assessing the role of BV on HIV-1 acquisition should control for HSV-2 infection in their statistical analyses

CONTRIBUTIONS

NN, PM and PV designed and coordinated the study. AO and NN implemented the study. Laboratory analyses were conducted by MCD under the supervision of PV. RV was responsible for the statistical analysis with NN and PM. The first draft of the manuscript was written by NN, PV and PM. All authors contributed significantly to the manuscript and approved the final version.

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