Distinguishing the Temporal Association between Women’s Intravaginal Practices and Risk of Human Immunodeficiency Virus Infection: A Prospective Study of South African Women

Landon Myer1,2, Lynette Denny3, Michelle de Souza4, Thomas C. Wright, Jr.5, and Louise Kuhn2,6

1 Infectious Diseases Epidemiology Unit, School of Public Health and Family Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa.
2 Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY.
3 Department of Obstetrics and Gynaecology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa.
4 School of Public Health and Family Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa.
5 Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, NY.
6 Gertrude H. Sergievsky Center, College of Physicians and Surgeons, Columbia University, New York, NY.

Received for publication September 6, 2005; accepted for publication October 31, 2005.

Cross-sectional studies have suggested that intravaginal practices, such as douching or “dry sex,” may increase women’s susceptibility to infection with human immunodeficiency virus (HIV). The authors examined the temporal nature of this association in a cohort of South African women. At enrollment (2001–2002), 4,089 women were tested for HIV infection. Participants reported their intravaginal practices at a 6-month follow-up visit and were followed with repeat HIV testing for up to 24 months. Among the 3,570 women who were HIV-negative at enrollment, 26% reported some type of intravaginal practice, mostly washing inside the vagina with water and/or cloth as part of daily hygiene. During follow-up, 85 incident HIV infections were observed. Intravaginal practices were associated with prevalent HIV at enrollment (adjusted odds ratio = 1.50, 95% confidence interval: 1.22, 1.85), but during follow-up there was no association between intravaginal practices and incident HIV (adjusted hazard ratio = 1.04, 95% confidence interval: 0.65, 1.68). These findings may be explained by a reversal of the causal sequence assumed for this association, since intravaginal practices may be undertaken in response to vaginal infections that occur more commonly among HIV-infected women. Intravaginal practices appear unlikely to be a cofactor in the male-to-female transmission of HIV in this setting.

HIV; risk factors; sexually transmitted diseases; South Africa; vaginal discharge; vaginal douching

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus.

Reports published over the past decade have described women’s intravaginal practices in different parts of Sub-Saharan Africa and other regions (1–4). These practices involve the insertion of various materials or substances into the vagina, including liquids, paper, cloth, and medicinal preparations. In the context of human immunodeficiency virus (HIV) infection, intravaginal practices first gained attention among sex workers seeking to remove moisture prior to intercourse, a practice referred to as “dry sex” (5, 6). Subsequent investigations have shown that these behaviors take place for a range of different purposes, including the removal of vaginal discharge and/or regular hygiene (2, 7). Research
from different parts of Africa has suggested that intravaginal practices may be relatively common in general population samples, with reported prevalences ranging from 20 percent to 50 percent (7–13); slightly higher prevalences have been documented among female sex workers (5, 14–16).

Recent research has suggested an association between intravaginal practices and women’s susceptibility to HIV infection. Several cross-sectional studies have found significant associations between intravaginal practices and prevalent HIV infection (1, 5, 8, 13, 17). However, cross-sectional data are unable to distinguish the temporal sequence of this association—that is, whether intravaginal practices increase susceptibility to HIV infection or whether HIV infection leads to increased intravaginal practices. The latter possibility requires careful consideration, because a range of different vaginal infections, such as Trichomonas vaginalis and Candida species, appear more commonly among HIV-infected women (18, 19). It is plausible that intravaginal practices may be undertaken to help relieve the symptoms of vaginal infection, including discharge and/or pruritus. There is also substantial evidence showing that intravaginal practices are positively correlated with different behavioral risk factors for HIV (e.g., number and type of sexual partners) (7, 20–22). As a result, the prospect of confounding by sexual behaviors requires particular attention.

Despite the importance of discerning the temporality of any association involving intravaginal practices and women’s risk of HIV infection, there have been few prospective studies investigating this question. One Zambian study of 634 women visiting postnatal clinics found a substantially higher risk (relative risk = 38.0) of incident HIV among women reporting intravaginal practices, but this association was not adjusted for any potentially confounding variables (17). Another study of 500 sex workers in Thailand showed no association (relative risk = 1.1) between risk of HIV seroconversion and the use of policresulen vaginal suppositories (23). However, these suppositories are not widely used (24), and thus it is unclear how these results may be generalized to other types of intravaginal practices. Other prospective studies of intravaginal practices have not evaluated risk of HIV infection as an outcome but have focused instead on other sexually transmitted infections and vaginal epithelial disruptions (25, 26).

If intravaginal practices truly increase women’s susceptibility to HIV infection, these behaviors may be an important target for HIV prevention interventions. However, given the limited amount of prospective data, additional data are clearly required to establish the nature of this association before any intervention strategy can be recommended. We investigated intravaginal practices and risk of incident HIV infection in a cohort of women in Cape Town, South Africa. Previously, we observed a significant adjusted association between intravaginal practices and prevalent HIV in this population (27). This analysis was undertaken to evaluate the temporal nature of the cross-sectional finding.

**MATERIALS AND METHODS**

This study was nested within a larger randomized controlled trial evaluating various cervical cancer screening modalities in the African community of Khayelitsha, outside of Cape Town, South Africa (28). Women were recruited into the trial through community meetings and by health workers advertising a cancer prevention service. Volunteers were eligible if they: were between the ages of 35 and 65 years; had not previously had a Papanicolaou smear or treatment for cervical neoplasia; did not have any clinical signs of cervical cancer (including a four-quadrant high-grade squamous intraepithelial lesion); and were suitable for cryotherapy (e.g., had a cervix and did not have cervical polyps or prolapse). These criteria ruled out 6 percent of women who volunteered to participate, primarily because they were unsuitable for cryotherapy. All participants provided written informed consent before being enrolled in the study. Ethical approval for the study was received from the Institutional Review Board of Columbia University (New York, New York) and the Research Ethics Committee of the University of Cape Town (Cape Town, South Africa).

Upon enrollment in the trial, women underwent clinical examination, completed a questionnaire on demographic characteristics and sexual behaviors, and provided blood samples for HIV testing. Samples for Neisseria gonorrhoeae and Chlamydia trachomatis were collected using endocervical cone-brushes and were tested using the Hybrid Capture GC/CT DNA Assay (Digene Corporation, Gaithersburg, Maryland). Endocervical cone-brush samples were also tested for human papillomavirus DNA using the Hybrid Capture II HPV DNA Assay (Digene Corporation). Wet-mount examinations were performed on-site during the clinical examination by trained study nurses to identify T. vaginalis. Bacterial vaginosis was assessed during the clinical examination and was considered present if at least three of the following four signs were noted: moderate or severe vaginal discharge; a positive whiff test after application of potassium hydroxide; the presence of clue cells upon wet-mount examination; and vaginal pH greater than 4.5. Blood specimens taken at each study visit were tested for HIV antibodies using the Abbott HIV 1/2 g 0 Kit on the Abbott AXSYM system (Abbott Laboratories, Abbott Park, Illinois). Positive results were confirmed using the Vironosticka HIV Uniform 2 Plus 0 Kit (Organon Teknika, Boxtel, the Netherlands). All HIV results were recorded using an anonymous, linked system, and separate voluntary counseling and testing services were provided for participants wishing to know their HIV serostatus.

At a follow-up visit 6 months later, participants provided blood samples for HIV testing and completed a questionnaire which included detailed questions about intravaginal practices and sexual behaviors. This instrument was administered by trained study nurses and was designed for maximum sensitivity in detecting any type of intravaginal practice, including “dry sex” practices, douching or washing inside the vagina with liquids, and the insertion of any substances into the vagina. Because of concerns about the measurement of intravaginal practices, we conducted qualitative research (in-depth interviews) with women in this setting before the study began in order to investigate the different types of intravaginal practices that were known in this setting and the most appropriate terminology for referring to these practices. From this research, we developed
a preliminary study instrument that was pilot-tested over a 2-month period. The results of this pilot study were used to refine the questionnaire structure and item phrasing for the final study instrument. This instrument investigated 15 separate types of intravaginal practices (identified during qualitative research and the pilot study) and then employed open-ended questions to identify other practices that had not been specifically asked about. Before asking questions on intravaginal practices, study nurses were trained to clearly distinguish intravaginal practices from wiping or cleaning of the external genitalia only. In addition to the type of practice and the substances involved, the questionnaire collected data on the reasons for women’s use of intravaginal products, the frequency of various practices, and their proximity to sexual intercourse.

Between March 2001 and December 2002, 4,668 women were enrolled in the study. Of these, 4,099 women (88 percent) completed the 6-month follow-up visit including the questionnaire on intravaginal practices; 4,089 had HIV test results available at baseline and at 6 months and were eligible for this analysis. All women were scheduled to be followed for at least 6 months within the cervical cancer trial \((n = 4,089)\). In addition, a subset of participants who either 1) were found to be positive upon direct visual inspection of the cervix at enrollment, 2) tested positive for human papillomavirus DNA at enrollment, and/or 3) were enrolled after January 2002 were scheduled by design to receive extended follow-up for 24 months after enrollment as part of a cervical cancer substudy \((n = 2,674)\). Thus, calculations of person-time and loss to follow-up in this analysis were based on women followed for 6 months and the subset of women scheduled for additional follow-up for 24 months. All 24-month follow-up visits were completed (or participants were declared lost to follow-up) by March 2005.

Data were analyzed using the statistical program SAS, version 9.0 (SAS Institute, Inc., Cary, North Carolina). We conducted separate analyses of findings based on HIV incidence from the 6-month follow-up visit (including the entire study cohort), as well as the substudy cohort followed for 24 months; since no substantive differences were noted, we report only the results obtained at 24 months. Women’s intravaginal practices were categorized according to their degree of hypothesized abrasiveness to vaginal epithelium, with a basic distinction between less abrasive practices (involving water, paper, and/or cloth only) and the insertion of other, potentially more abrasive products inside the vagina. Incident HIV infection was defined as a negative test result followed by a positive result at the next study visit, and dates of seroconversion were estimated using the midpoint of this interval (29). HIV seroconversion probabilities were compared among subgroups of the study sample using Kaplan-Meier methods. Proportional hazards regression models were used to estimate the association between selected covariates and HIV seroconversion. In addition, we repeated our previous analyses of the association between prevalent HIV infection and intravaginal practices using multiple logistic regression (27). For both proportional hazards and logistic regression models, variables were included in the analysis if they demonstrated an appreciable crude association with HIV infection; variables were removed from the analysis if they showed no adjusted association with HIV and their removal did not alter the associations involving other covariates. The proportional hazards regression results are reported as hazard ratios with 95 percent confidence intervals. All statistical tests were two-sided.

RESULTS

At enrollment, 519 (13 percent) of the 4,089 women with available data on intravaginal practices tested positive for HIV infection. Women with prevalent HIV infection at enrollment were significantly more likely to report intravaginal practices than were women testing negative: 17 percent of women reporting intravaginal practices were HIV-infected as compared with 11 percent of women not reporting intravaginal practices (crude odds ratio \(= 1.61, 95\) percent confidence interval \(\text{CI}: 1.33, 1.95\)). This association persisted after adjustment for age, education, lifetime number of sexual partners, recent sexual activity, and prevalent infection with human papillomavirus, trichomonas, and chlamydia and/or gonorrhea (adjusted odds ratio \(= 1.50, 95\) percent CI: 1.22, 1.85). Prevalent HIV infection was strongly associated with an increasing amount of vaginal discharge, with no vaginal discharge being evident during clinical examination of 17 percent of HIV-negative women and 12 percent of HIV-positive women, while moderate or severe discharge was noted among 20 percent and 28 percent of HIV-negative and -positive women, respectively \(p_{\text{trend}} < 0.001\).

A total of 4,641 person-years of observation were accrued among the 3,570 women who tested HIV-negative at enrollment. Within the cohort designated for follow-up beyond the 6-month visit, follow-up rates for subjects who were HIV-negative at enrollment were 83 percent \((n = 1,887)\) and 75 percent \((n = 1,718)\) at the 12- and 24-month visits, respectively. Over the course of follow-up, 83 participants missed a scheduled study visit but attended the following visit. Persons lost to follow-up were slightly younger than those who were successfully followed \(\text{mean age} = 43.4\) years vs. 44.7 years; \(p = 0.01\), but those lost did not differ from those followed in terms of education, marital status, housing type, intravaginal practices, sexual activity, or number of sexual partners at enrollment, or by baseline prevalence of bacterial vaginosis, trichomoniasis, or chlamydia and/or gonorrhea (data not shown).

Baseline demographic characteristics, sexual behaviors, and prevalent sexually transmitted diseases among women who were HIV-negative at enrollment are described in table 1. The median age was 42 years (interquartile range, 38–48); slightly more than half of the participants were married; and most of the women \(70\) percent; \(n = 2,492\) lived in shacks or informal housing (squatter camps), an indicator of low socioeconomic status. Although most women had been sexually active during the month prior to the study \(72\) percent; \(n = 2,588\), 98 percent reported having had only one sexual partner during this period \(n = 2,541\), and condom use was low.

Among women who tested HIV-negative at enrollment, 26 percent reported some type of intravaginal practice at
the 6-month follow-up interview (n = 945). The majority (63 percent; n = 592) of these women described potentially less abrasive practices, primarily involving the insertion of water, paper, and/or cloth only inside the vagina (table 2). Within this group, 453 women reported practices involving cloth specifically. Among the 348 women reporting potentially more abrasive practices, the most common products inserted into the vagina included household or laundry soaps (n = 158; 17 percent of all women reporting intravaginal practices), household antiseptics (n = 121; 13 percent), vinegar (n = 18; 2 percent), salt or saltwater (n = 19; 2 percent), and industrial detergents (n = 14; 1 percent) (participants could report using more than one product).

The majority of women reporting intravaginal practices stated that these activities took place as part of regular washing (88 percent; n = 835), while smaller proportions reported engaging in these practices before or after sexual intercourse (6 percent; n = 60) or in association with menses (5 percent; n = 50). Only nine women (1 percent) reported engaging in intravaginal practices prior to intercourse. Slightly more than half reported engaging in these practices daily (51 percent; n = 486), and 75 percent reported undertaking intravaginal practices during the week prior to being interviewed (n = 713). The majority of women gave hygiene as the motivation for their intravaginal practices (69 percent; n = 650), with a lower proportion giving removal of excess vaginal discharge as their primary reason (21 percent; n = 202). There were no associations between the reasons given for intravaginal practices and the specific practices involved (data not shown).
unadjusted hazard ratio = 1.11, 95 percent CI: 0.69, 1.78), nor were more abrasive practices related to seroconversion (table 2). In one small subgroup of less abrasive practices—wiping inside the vagina with fingers, with or without water—the incidence rate of HIV infection was significantly higher than that among women reporting no intravaginal practices (crude hazard ratio = 3.66, 95 percent CI: 1.82, 7.37). However, within all of the other subgroups, there were no significant differences between women in subgroups of intravaginal practices and women reporting no intravaginal practices.

Other factors associated with incident HIV infection in unadjusted analyses included younger age, being unmarried, trichomons infection at enrollment, and chlamydia and/or gonorrhea infection at enrollment (table 3). In stratified analyses, the null association between intravaginal practices and incident HIV infection persisted across all levels of these factors.

In a proportional hazards regression model adjusting for demographic characteristics, sexual behaviors, and prevalent sexually transmitted infections at baseline, the overall null association between intravaginal practices and HIV incidence persisted (hazard ratio = 1.04, 95 percent CI: 0.65, 1.68) (table 4). Increased hazards of incident HIV infection were associated with being unmarried and being younger. In another model (data not shown) containing separate terms for women reporting less abrasive practices and women reporting more abrasive practices (compared with women reporting no practices), there was no adjusted association between less abrasive practices and incident HIV infection (hazard ratio = 0.71, 95 percent CI: 0.29, 1.77).

DISCUSSION

This was a large prospective study investigating intravaginal practices as a risk factor for incident and prevalent HIV infection in a general population sample of women. Our results demonstrate an adjusted association between intravaginal practices and prevalent HIV infection, in keeping with a previous analysis (27), but no association between intravaginal practices and incident HIV infection. This evidence may appear at first to be contradictory, with the cross-sectional result suggesting that intravaginal practices may...
increase women’s susceptibility to HIV infection and the prospective data suggesting no association. However, taken together, these results may be explained by a reversal of the causal sequence commonly assumed for this association. Specifically, intravaginal practices in this setting may increase as a result of women’s HIV infection.

Two aspects of these data support this finding. First, the vast majority of women who reported intravaginal practices stated that these behaviors were part of their regular hygiene or were done to relieve symptoms of itching or excess discharge. While we found no difference in intravaginal practices according to degree of vaginal discharge at enrollment,

**TABLE 3. Crude probabilities of human immunodeficiency virus (HIV) seroconversion according to selected risk factors and associated hazard ratios among 3,570 HIV-negative women in Cape Town, South Africa, March 2001–March 2005**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>No. of incident infections</th>
<th>No. of women reporting</th>
<th>Probability of seroconversion</th>
<th>Log-rank p value*</th>
<th>Crude hazard ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35–39</td>
<td>43</td>
<td>1,345</td>
<td>0.049</td>
<td></td>
<td>1.0†</td>
<td></td>
</tr>
<tr>
<td>40–49</td>
<td>35</td>
<td>1,537</td>
<td>0.035</td>
<td>0.010</td>
<td>0.688</td>
<td>0.440, 1.075</td>
</tr>
<tr>
<td>50–65</td>
<td>7</td>
<td>688</td>
<td>0.019</td>
<td>0.004</td>
<td>0.569</td>
<td>0.381, 0.848</td>
</tr>
<tr>
<td>Completed primary education</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>38</td>
<td>1,623</td>
<td>0.036</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>47</td>
<td>1,947</td>
<td>0.038</td>
<td>0.974</td>
<td>1.007</td>
<td>0.657, 1.545</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not married</td>
<td>55</td>
<td>1,692</td>
<td>0.049</td>
<td></td>
<td>1.0†</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>30</td>
<td>1,878</td>
<td>0.026</td>
<td>0.002</td>
<td>0.493</td>
<td>0.316, 0.769</td>
</tr>
<tr>
<td>Housing type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal</td>
<td>24</td>
<td>1,078</td>
<td>0.031</td>
<td></td>
<td>1.0†</td>
<td></td>
</tr>
<tr>
<td>Informal/shack</td>
<td>61</td>
<td>2,492</td>
<td>0.040</td>
<td>0.506</td>
<td>1.174</td>
<td>0.732, 1.882</td>
</tr>
<tr>
<td>New sexual partner in past 5 months‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>80</td>
<td>3,470</td>
<td>0.037</td>
<td></td>
<td>1.0†</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>100</td>
<td>0.063</td>
<td>0.076</td>
<td>2.217</td>
<td>0.898, 5.473</td>
</tr>
<tr>
<td>Partner had sex with other women in past 5 months‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>17</td>
<td>907</td>
<td>0.032</td>
<td></td>
<td>1.0†</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>24</td>
<td>741</td>
<td>0.047</td>
<td>0.077</td>
<td>1.739</td>
<td>0.935, 3.236</td>
</tr>
<tr>
<td>Don’t know</td>
<td>29</td>
<td>1,141</td>
<td>0.043</td>
<td>0.259</td>
<td>1.410</td>
<td>0.775, 2.565</td>
</tr>
<tr>
<td>No partner</td>
<td>15</td>
<td>781</td>
<td>0.027</td>
<td>0.909</td>
<td>1.020</td>
<td>0.721, 1.444</td>
</tr>
<tr>
<td>Baseline trichomonas infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>72</td>
<td>3,244</td>
<td>0.035</td>
<td></td>
<td>1.0†</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>326</td>
<td>0.064</td>
<td>0.040</td>
<td>1.837</td>
<td>1.018, 3.317</td>
</tr>
<tr>
<td>Baseline clinical evidence of bacterial vaginosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>60</td>
<td>2,843</td>
<td>0.033</td>
<td></td>
<td>1.0†</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25</td>
<td>727</td>
<td>0.053</td>
<td>0.055</td>
<td>1.573</td>
<td>0.986, 2.508</td>
</tr>
<tr>
<td>Baseline chlamydia and/or gonorrhea infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>76</td>
<td>3,402</td>
<td>0.035</td>
<td></td>
<td>1.0†</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>9</td>
<td>168</td>
<td>0.085</td>
<td>0.011</td>
<td>2.396</td>
<td>1.201, 4.782</td>
</tr>
</tbody>
</table>

* Seroconversion (failure) probabilities and log-rank tests were from Kaplan-Meier analyses.
† Reference category.
‡ This information was obtained at the 6-month follow-up visit.
women entering the study with prevalent HIV infection were significantly more likely to have moderate or severe vaginal discharge than women without HIV (27). In this light, it is plausible that HIV infection over time causes or exacerbates lower genital tract infections as immunosuppression increases, leading to increased vaginal discharge and related symptoms, which in turn motivate intravaginal practices. This is supported by data demonstrating an increased prevalence of discharge-producing infections among HIV-positive women, including vulvovaginal candidiasis, trichomonas, and bacterial vaginosis (19, 30, 31), as well as data showing that increased disease progression is linked to increased incidence and persistence of vaginal candidiasis (32) and bacterial vaginosis (33). Second, intravaginal practices prior to sexual intercourse appeared to be uncommon in this population, suggesting that these behaviors were not temporally associated with sexual intercourse and possible exposure to HIV infection. The potential for intravaginal practices to disrupt mucosal membranes prior to sex is the most commonly hypothesized mechanism for intravaginal practices’ increasing susceptibility to HIV (16); other mechanisms of action that may not require a close temporal association with sexual intercourse remain unclear.

While there have been few prospective investigations of intravaginal practices and HIV infection, data from research carried out among Thai sex workers (34) appear to be broadly consistent with our findings. A preliminary cross-sectional study of these brothel-based sex workers found a link between intravaginal practices and prevalent HIV infection (34), suggesting that these behaviors may increase women’s susceptibility to HIV. However, later investigations revealed no association with incident HIV infection (23, 35). Taken together, these results point to similar interpretations: that intravaginal practices may be motivated by factors related to HIV infection, rather than these behaviors’ increasing women’s risk of becoming infected with HIV.

In addition to the hypothesis that HIV infection leads to increased intravaginal practices, there are several alternative explanations for the observed data. First, if intravaginal practices do truly increase women’s risk of HIV infection, the disagreement between the prevalent results and the incident results could be explained by the highest-risk women’s being lost to follow-up. However, the rates of follow-up in this cohort were relatively high, and there were few systematic differences between the women retained in the cohort and those who were lost to follow-up. To evaluate the possibility of differential loss to follow-up as a possible explanation for the observed data, we conducted a basic sensitivity analysis (data not shown). This analysis suggested that in order to explain these study results, persons lost to follow-up would have required at least twice the HIV incidence of those retained in the cohort and would have had to be more than five times as likely to report intravaginal practices as those retained in the cohort. Neither of these parameters is consistent with the available data, which show that women lost to follow-up were slightly younger (mean difference in age = 1.3 years) than those retained but did not differ from those retained according to any other measured variable, including reported intravaginal practices. In this light, differential loss to follow-up is unlikely to have played a significant role in explaining the differences between incident and prevalent results. Related to this, there were no associations between any of the criteria for inclusion in the substudy cohort followed to 24 months and either women’s intravaginal practices or incident HIV infection, suggesting that the possibility of selection bias is unlikely to explain these findings.

Second, with 85 seroconversions, this analysis had reasonable statistical power to detect associations of the magnitude observed for intravaginal practices and prevalent HIV. Along these lines, the 95 percent confidence interval for the adjusted odds ratio for the prevalent association (95 percent CI: 1.20, 1.82) was not consistent with the adjusted hazard ratio for the incident association (hazard ratio = 1.04), suggesting that random sampling error is unlikely to account for these differences.

Third, it may be possible that unmeasured confounding created a spurious difference between prevalent and incident results. In particular, we did not have detailed information on high-risk sexual behaviors occurring throughout the follow-up period, and we were able to adjust for new sexual partners only at the 6-month follow-up visit (by which time the majority of seroconversions had taken place). We also did not have data on the duration and nature of menses.
which could be correlated with intravaginal practices and could act as a confounder of the observed associations. While some unmeasured confounding may be plausible, we did examine a number of potential demographic, behavioral, and biologic confounding variables. It is not clear what biologic or behavioral variables could act as confounders of either the cross-sectional analysis or the prospective analysis but not both, and thus help to explain the observed associations.

We measured intravaginal practices only at the 6-month follow-up visit, and we used this measure to reflect behaviors occurring throughout the follow-up period. While our preliminary qualitative research (described above) suggested that these behaviors were stable through time, it is possible that intravaginal practices fluctuated for some women over the course of the study, introducing some degree of measurement error into the analysis. Similarly, the reporting of intravaginal practices may be awkward for women and thus subject to some degree of systematic reporting error. However, in order for either form of misclassification in the assessment of intravaginal practices to create a bias that would explain the observed discrepancy between incident and prevalent findings, seroconverters in the prospective study of HIV would need to substantially underreport their intravaginal practices, relative to women who would not seroconvert in the prospective study and all women in the cross-sectional study (36). In this light, it seems unlikely that these forms of mismeasurement could systematically affect the incident analysis but not the prevalent analysis.

Given the wide variation in intravaginal practices reported around the world, the generalizability of these findings should be viewed critically. Although they were still at considerable risk of HIV infection, this sample of women was substantially older (mean age = 42 years) than the populations examined in other studies. More importantly, there is variability across populations in the motivations for and types of intravaginal practices. Although the forms of wiping and/or douching with cloth and/or water for hygienic purposes that appeared to be most common in this study population have been documented in several other settings (7, 12, 26), other behaviors appear to be more common in samples of women from different countries (1, 10). Intravaginal practices designed to remove vaginal moisture before intercourse (“dry sex”), which have been the focus of several previous studies, appeared to be uncommon in this population. The relation between intravaginal practices and HIV infection may vary with the specific practices involved, the reasons for those practices, and their proximity to sexual intercourse; as a result, our overall interpretation may not be applicable to all types of intravaginal practices in all places.

In summary, these results indicate that previous cross-sectional studies may have overestimated the association between women’s intravaginal practices and HIV infection. The types of intravaginal practices reported in this study population appear unlikely to be an important cofactor in the spread of HIV. Instead, existing HIV infection may motivate increased intravaginal practices, spuriously inflating the association between intravaginal practices and prevalent HIV. Targeting intravaginal practices for HIV prevention is unlikely to be a useful strategy for reducing new infections in this setting.

ACKNOWLEDGMENTS

This research was funded by the Bill and Melinda Gates Foundation and EngenderHealth.
Conflict of interest: none declared.

REFERENCES


