Vaginal washing and increased risk of HIV-1 acquisition among African women: a 10-year prospective study

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\textbf{Background:} No prospective study has examined the risk of HIV-1 acquisition associated with vaginal washing, although intravaginal practices have been identified as potentially important contributors to HIV-1 susceptibility.

\textbf{Objective:} To evaluate the contribution of vaginal washing to incident HIV-1 infection.

\textbf{Design:} Prospective cohort study.

\textbf{Methods:} Data were derived from a 10-year study of risk factors for HIV-1 acquisition among 1270 Kenyan female sex workers. Intravaginal practices were ascertained at study enrollment. At monthly follow-up visits, women completed a standardized interview and specimens were collected for diagnosis of HIV-1 and genital tract infections.

\textbf{Results:} Compared with women who did not perform vaginal washing, there was an increased risk for acquiring HIV-1 among women who used water [adjusted hazard ratio (HR), 2.64; 95\% confidence interval (CI), 1.00–6.97] or soap (adjusted HR 3.84; 95\% CI, 1.51–9.77) to clean inside the vagina, after adjustment for demographic factors, sexual behavior, and sexually transmitted infections. Furthermore, women who performed vaginal washing with soap or other substances were at higher risk for HIV-1 compared with those who used water alone (adjusted HR, 1.47; 95\% CI, 1.02–2.13).

\textbf{Conclusions:} In populations where vaginal washing is common, this practice may be an important factor promoting the spread of HIV-1. Intervention strategies aimed at modifying intravaginal practices should be evaluated as a possible female-controlled HIV-1 prevention strategy.


\textbf{Keywords:} vaginal washing, HIV-1 risk, African women
Introduction

Intravaginal practices including douching, wiping, and inserting substances into the vagina have been associated with a higher prevalence of HIV-1 in some cross-sectional studies [1]. These practices have long been suspected to be associated with increased HIV-1 susceptibility [2], and are widespread in different regions and populations throughout sub-Saharan Africa [3–6]. Frequent use of intravaginal practices has been reported among female sex workers in Nairobi, Kenya [3]; attendees at a sexually transmitted infections clinic in Bangui, Central African Republic [5]; pregnant women in Abidjan, Côte d’Ivoire [4]; and family planning, primary care, and postnatal clinic attendees in Harare, Zimbabwe [6]. To date, prospective data on the relationship between intravaginal practices and HIV-1 acquisition are lacking. The present study evaluated the risk of HIV-1 acquisition using data from a 10-year prospective study of Kenyan sex workers.

Methods

Population and procedures
HIV-1-seronegative female sex workers attending a municipal sexually transmitted infection clinic in Mombasa, Kenya were enrolled in an open cohort study of risk factors for HIV-1 acquisition. Study procedures have been detailed previously [7]. Women were asked about intravaginal practices at enrollment, including detailed questions about the substances and methods they used for vaginal washing. Women were also asked about intravaginal use of traditional substances such as herbs and drying agents.

At monthly follow-up visits, sexual behavior and contraceptive use were recorded and a blood sample was collected for HIV-1 screening. A physical examination including a pelvic speculum examination, with collection of specimens for laboratory diagnosis of sexually transmitted infections, was performed. Genital ulcer disease was defined by the presence of a vulvar, vaginal, or cervical epithelial disruption. The presence of yellow or greenish cervical discharge was defined as cervical mucopus. All women received risk reduction counseling, treatment for sexually transmitted infections, and free condoms. This research was approved by the Human Subjects Committees of the University of Washington and the University of Nairobi. All participants provided informed consent.

Serology and microbiology
Screening for HIV-1 was performed using an enzyme-linked immunosorbent assay (Detect-HIV, Biochem Immunosystems, Montreal, Canada). Positive specimens were confirmed using a second enzyme-linked immunosorbent assay (Recombigen, Cambridge Biotech, Galway, Ireland). Endocervical secretions were cultured on modified Thayer–Martin media for Neisseria gonorrhoeae. A Gram stain of endocervical secretions was examined by microscopy, and the number of polymorphonuclear leukocytes in three non-adjacent high-power fields was determined. Cervicitis was defined by the presence of an average polymorphonuclear leukocytes count ≥ 30 cells/high power field. A saline wet preparation of vaginal secretions was examined by light microscopy for identification of Trichomonas vaginalis and yeast. Bacterial vaginosis was evaluated by microscopy of a vaginal Gram stain [8].

Data analysis
Data analysis was performed using SPSS version 10.0 (SPSS, Chicago, Illinois, USA) and S-Plus 2000 (Mathsoft, Cambridge, Massachusetts, USA). All HIV-1-seronegative women who enrolled in the cohort and had at least one follow-up visit were considered for inclusion in this analysis. Visits that took place as part of two studies of the vaginal microbicide nonoxynol-9 were excluded in order to eliminate any influence of this product on HIV-1 risk or vaginal cleansing practices [9,10].

Univariate and multivariate Cox proportional hazards modelling was performed to assess the effect of vaginal washing on HIV-1 acquisition. Multivariate models were adjusted for potential confounding factors including demographic characteristics, sexual risk behavior, contraceptive methods, condom use, and the presence of sexually transmitted infections. Adjustments made were for baseline education (≤ 8 versus > 8 years), parity (≤ 2 versus > 2), and workplace (bar versus nightclub; bar work has been associated with increased HIV-1 risk in this population [7]). Models were also adjusted for the following time-dependent covariates: bacterial vaginosis, trichomoniasis, vaginal yeast infection, genital ulcer disease, mucopurulent cervical discharge, microscopic cervicitis, cervical gonorrhea, hormonal contraceptive use (no contraception or tubal ligation versus oral contraceptive pills versus depot medroxyprogesterone acetate versus Norplant versus intrauterine device), age (< 25, 25–29, 30–34, 35–39, ≥ 40 years), duration of prostitution (< 1, 2–4, 5–9, ≥ 10 years), number of sexual partners per week (≤ 1 versus > 1), sexual frequency per week (≤ 2 versus > 2), and condom use (< 100% versus 100%). Condom use was considered separately from other methods of contraception since many women used condoms for protection from sexually transmitted infections. Because the effect of hormonal contraception may persist after discontinuing or changing methods, women were considered to be exposed for 115 days after the last reported use, as we have done previously [7]. The effect of sexually transmitted infections was considered to persist for 60 days [7]. For the sexual behavior variables (number of sexual partners, sexual frequency, and percentage condom use) an average was...
calculated for each year of follow-up in order to capture average behavior over time. Dichotomous categories for continuous variables were defined by the median for the cohort.

**Results**

Between 1993 and 2003, a total of 1496 women were enrolled in the cohort, of whom 1270 (85%) returned for follow-up. Compared to those with follow-up, women who did not return were younger [median 24 years, interquartile range (IQR), 21–29 versus median 26 years, IQR, 22–31; \( P = 0.001 \)], had a shorter duration of prostitution [median 0.8 years (IQR, 0.1–2.0) versus 1.0 years (IQR, 0.2–3.0); \( P = 0.01 \)], more education [median 8 years (IQR, 7–11) versus 8 years (IQR, 6–10); \( P = 0.003 \)], and fewer pregnancies [median 1 (IQR, 1–2) versus 2 (IQR, 1–3); \( P = 0.05 \)]. Women who were lost to follow-up did not differ significantly from women who returned in terms of their vaginal washing practices, number of sex partners per week, sexual frequency, condom use, contraceptive use, or prevalence of genital tract infections (data not shown).

Among the 1270 women who returned for follow-up, 652 (51%) had been married at least once, but only 16 (1%) were currently married. They reported a median of 2 (IQR, 1–3) sexual contacts per week, and 62% reported consistent condom use. None reported injection drug use, and only three (<1%) practiced anal sex. The median duration of follow-up was 468 days (IQR, 126–1217), the median time between visits was 35 days (IQR, 28–60). A total of 2877 person-years of follow-up were accrued. HIV-1 seroconversion occurred in 222 women (7.7/100 person-years).

Data on vaginal washing practices were collected at the enrollment visit: 71 (6%) women reported no vaginal washing, 293 (23%) reported using water only, and 906 (71%) reported using soap or other substances including detergents (<5%) and antiseptics (<5%). Only 15 (1%) women reported placing herbs or other substances in the vagina. The most common vaginal washing method was with a finger (998 women, 79%). Most of the remaining women reported using a piece of cloth (187, 15%), while only one (<1%) reported using a douching bag for vaginal washing.

Compared with women who did not perform vaginal washing, those who used water had a nearly three-fold increased risk of HIV-1 seroconversion, while those who used soap had an approximately four-fold increased risk (Table 1). A significantly higher risk of HIV-1 acquisition was present throughout the 10-year study period (Fig. 1), and this remained after adjustment for demographic factors, sexual behavior, and sexually transmitted infections. Moreover, women who used soap or other substances were at significantly higher risk for HIV-1 acquisition compared with women who used water alone [adjusted hazard ratio (HR), 1.47, 95% confidence interval (CI), 1.02–1.97].

Although data on vaginal washing were initially only collected at the enrollment visit, beginning in 1998,

![Table 1. HIV-1 incidence among women with different vaginal washing practices.](image)

<table>
<thead>
<tr>
<th>Vaginal washing practice</th>
<th>None</th>
<th>Water only</th>
<th>Soap/other&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. women</td>
<td>71</td>
<td>293</td>
<td>906</td>
</tr>
<tr>
<td>No. HIV-1 seroconversions</td>
<td>5</td>
<td>45</td>
<td>172</td>
</tr>
<tr>
<td>HIV-1 incidence (per 100 person-years)</td>
<td>1.84</td>
<td>5.88</td>
<td>9.35</td>
</tr>
<tr>
<td>Hazard ratio (95% confidence interval)</td>
<td>1.0</td>
<td>2.75 (1.06–7.10)</td>
<td>4.03 (1.61–10.10)</td>
</tr>
<tr>
<td>( P ) value</td>
<td>0.04</td>
<td>0.003</td>
<td>0.03</td>
</tr>
<tr>
<td>Adjusted hazard ratio (95% confidence interval)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0</td>
<td>2.64 (1.00–6.97)</td>
<td>3.84 (1.51–9.77)</td>
</tr>
<tr>
<td>( P ) value</td>
<td>0.05</td>
<td>0.005</td>
<td>0.005</td>
</tr>
</tbody>
</table>

<sup>a</sup>Multivariate models adjusted as described in the text.<br>
<sup>b</sup>Less than 5% of women used detergent or antiseptic.
Discussion

This study demonstrated a stepwise increase in the risk of HIV-1 acquisition when women who did not perform vaginal washing were compared with women who used water alone and with women who used soap or other substances to clean inside the vagina. To our knowledge, this is the first prospective study to demonstrate a significant association between vaginal washing and HIV-1 acquisition. The prospective cohort design and careful adjustment for potential confounding factors support these findings as the strongest evidence to date that intravaginal practices, particularly vaginal washing with soap, may increase HIV-1 risk.

A causal association between vaginal washing and HIV-1 acquisition seems biologically plausible. Vaginal cleansing could disrupt the genital mucosa or cause inflammation, increasing HIV-1 risk. Intravaginal practices could also increase susceptibility to the virus by disrupting normal vaginal flora, decreasing colonization with Lactobacillus species, which may be protective against HIV-1 [7,11], and increasing vaginal pH. These practices could also indirectly increase the risk of HIV-1 acquisition by increasing susceptibility to genital tract infections [7]. However, it is notable that the associations between vaginal washing and HIV-1 seroconversion found in this study remained significant even in adjusted analyses that controlled for the presence of sexually transmitted infections, bacterial vaginosis, and vulvovaginal candidiasis. This observation suggests that vaginal washing might increase HIV-1 susceptibility at least partially through a mechanism that is independent of genital tract infections.

More than 20 years into the HIV-1 pandemic there is still no effective vaccine and few strategies have been proven to reduce the risk of HIV-1 acquisition in randomized clinical trials [12]. While condom use, mutual monogamy with a seronegative partner, and abstinence decrease risk, there remains an urgent need for novel interventions that can prevent HIV-1 transmission to women, who account for the majority of new infections. Several lines of evidence suggest that interventions to modify intravaginal practices should be considered as a potentially important strategy for developing a female-controlled method for HIV-1 prevention. First, these practices are associated with a substantial increase in HIV-1 risk. Indeed, the magnitude of the HIV-1 risk associated with vaginal washing in this study was comparable to the risk that has been associated with non-ulcerative genital tract infections [7]. Second, these practices are highly prevalent, having been reported in over one-third of women from various clinical settings and regions in sub-Saharan Africa [3,4,6,13]. Third, there is evidence that intravaginal practices can be modified. For example, microbicide trials have shown that women in high-risk settings are willing to modify intravaginal practices if they believe that doing so may decrease their HIV-1 risk [9]. Moreover, a recent randomized trial demonstrated that individualized stage-specific interventions can significantly reduce the proportion of women who perform vaginal washing and that this effect can persist for at least 1 year [14]. A thorough understanding of the norms and beliefs surrounding the use of intravaginal practices in various populations will be needed in order to develop culturally appropriate intervention strategies.

The prospective cohort design used in this study has limitations. Despite rigorous adjustment, it is possible that residual confounding could contribute to the observed association between vaginal washing and HIV-1 seroconversion. Although we adjusted for sexually transmitted infections and number of sexual partners using time-dependent analyses, these do not allow us to determine whether vaginal washing practices were different in response to partner types (e.g. casual clients versus more regular partners such as boyfriends), perceived higher-risk partners, or recent sexually transmitted infections. Our main analysis used vaginal washing practices reported at study enrollment rather than at follow-up visits, and thus may have better captured usual washing behaviors and been less subject to bias owing to recent sexual behavior or genital tract symptoms. In addition, this study did not include a qualitative evaluation of the reasons for vaginal washing. Behavioral research will be important to improve understanding of the social and cultural factors that underlie these practices. Randomized trials of interventions to modify intravaginal practices could provide definitive evidence of a causal association between vaginal washing and HIV-1 acquisition.

In conclusion, vaginal washing was highly prevalent in this population of African women and was associated with increased risk for acquiring HIV-1. Furthermore, vaginal washing with soap or other substances was associated with a higher risk of HIV-1 seroconversion than vaginal washing with water alone. Where intravaginal practices are widespread, even a modest increase in susceptibility could lead to a high population attributable fraction of HIV-1 infection associated with these practices.

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References