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Lower levels of HIV-2 than HIV-1 in the female genital tract: correlates and longitudinal assessment of viral shedding

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Abstract

Background—The differing magnitude of the HIV-1 and HIV-2 epidemics is likely a consequence of differing transmission rates between the two viruses. Similar to other sexually transmitted pathogens, risk of HIV-1 and HIV-2 transmission is likely associated with the presence and amount of HIV in the genital tract. Thus, understanding patterns of, and risk factors for HIV genital tract shedding is critical to effective control of HIV transmission.

Methods—We evaluated HIV DNA and RNA detection in cervicovaginal specimens among 168 HIV-1 and 50 HIV-2-infected women in Senegal, West Africa. In a subset of 31 women (20 with HIV-1, 11 with HIV-2), we conducted a prospective study in which cervicovaginal specimens were taken at 3-day intervals over a 6-week period.

Results—We found significantly lower rates and levels of HIV-2 RNA (58% shedding; 13% with >1000 copies/ml) in the female genital tract than HIV-1 RNA (78% shedding; 40% with >1000 copies/ml) ($P=0.005$ and 0.005 , respectively), and shedding correlated with plasma viral load irrespective of virus type (odds ratio =1.9, 95% confidence interval =1.3–2.8 for each log₁₀ increase in HIV viral RNA). Plasma viral load, not HIV type, was the strongest predictor of genital viral load. Over 80% of closely monitored women, regardless of HIV type, had at least intermittent HIV RNA detection during every 3-day sampling over a 6-week time period.

Conclusion—These data help in explaining the different transmission rates between HIV-1 and HIV-2 and may provide new insights regarding prevention.

Keywords

Africa; cervix; women; HIV-1; HIV-2; Senegal; vagina; virus shedding

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S.E.H. was primarily responsible for the writing of the manuscript, supervised data management of the project, and oversaw the statistical analysis. P.S.S. coordinated recruitment of participants in Senegal and oversaw patient care. J.E.S. conducted the data analysis. G.S.G. provided insight into interpretation of the study data and critical revisions of the manuscript. N.B.K. and C.W.C. were responsible for conception of the study, acquisition of funding, and supervised the project from the inception. All authors contributed to the initial draft and final manuscript.

Introduction

The pandemic spread of HIV-1, in contrast to the limited spread of HIV-2, primarily in West Africa, is likely due to different rates of transmission of these two viruses [1,2]. Nonetheless, both HIV-1 and HIV-2 are primarily spread by heterosexual transmission. Similar to other sexually transmitted infections, risk of HIV transmission is in part a function of the quantity of HIV in genital tract secretions [3–9]. A greater understanding of patterns and risk factors associated with genital tract HIV-1 and HIV-2 shedding will permit the development of more efficient strategies to control HIV transmission [10]. Only two studies have compared risk factors for detecting HIV-1 versus HIV-2 in the female genital tract; both were cross-sectional. Ghys *et al.* found genital HIV DNA in 24 and 5% of female commercial sex workers with HIV-1 and HIV-2, respectively [11], and Seck *et al.* [12] found cell-free HIV RNA in 8 and 6% of female commercial sex workers with HIV-1 and HIV-2, respectively, and in 41 and 33% of HIV-1 and HIV-2-infected women who were not sex workers. Potential differences in HIV shedding rates among patients infected with HIV-1 versus HIV-2 have been hypothesized to result from lower HIV plasma loads among HIV-2-infected persons [13–15]. Indeed, HIV-1 plasma viral load has been the only factor directly linked to transmission [16,17]. However, as direct transmission studies are often logistically difficult, genital shedding is often used as a surrogate marker. Factors shown to be associated with genital tract HIV-1 RNA or DNA detection in African studies include systemic factors such as high HIV RNA plasma load [12,18], CD4 cell count depletion [4,11,18,19], local (genital tract) factors such as elevated vaginal pH [12], genital ulceration [11], and cervical inflammation or genital infection [4,11,20]. In the one study [12] examining correlates of female genital HIV-2 shedding, low CD4 cell count was found to be the only independent risk factor. In men, HIV shedding in semen is at lower levels in HIV-2 as compared with HIV-1, and is strongly associated with plasma viral load [21].

In this study, we examined the prevalence, levels, and correlates of genital tract shedding in HIV-1 and HIV-2-infected women from Senegal, West Africa.

Patients and methods

Study population and study design

In order to comprehensively assess HIV shedding in the genital tract in women with HIV-1 or HIV-2, we conducted both cross-sectional and prospective studies of HIV DNA and RNA detection in cervicovaginal specimens among HIV-infected women in Senegal, West Africa. Between September 1994 and July 1998, women older than 15 years presenting to the University of Dakar Fann Hospital Infectious Disease Clinic were offered HIV-1 and HIV-2 serologic testing. Sera were tested using a microwell plate HIV-1/HIV-2 enzyme immunoassay (Genie; Sanofi Diagnostic Pasteur, Marnes La Coquette, France), with positive sera confirmed using a rapid synthetic peptide-based membrane immunoassay (Multispot; BioRad Laboratories, Hercules, California, USA), which classifies patients as HIV-1, HIV-2, or dually seropositive. From among 4119 women screened, 335 women with HIV-1, 69 with HIV-2 and 29 with dual HIV-1 and HIV-2 infection were identified, of which 237, 57 and 21, respectively, were enrolled into a cohort study assessing the effect of HIV on risk of cervical neoplasia [22]. Baseline cervicovaginal samples for HIV detection assays were obtained from 168 enrolled women infected with HIV-1 and 50 with HIV-2; women with dual HIV infection are not included in the current analysis. In addition, between February and August 1996, a subset of 31 women (20 with HIV-1 and 11 with HIV-2) with baseline samples participated in a substudy in which cervicovaginal wash specimens were taken at 3-day intervals over a 6-week period. Within virus type, women with cervicovaginal samples were similar to women without samples in the overall cohort

with respect to age, marital status, parity, birth control method, and CD4 cell count. As this study was conducted prior to the availability of HAART in Senegal, none of the patients were receiving antiretroviral therapies (ARTs). This study was conducted according to procedures approved by the Institutional Review Boards of the Universities of Washington and Dakar, and the Senegalese National AIDS Committee. All patients provided informed consent for study participation.

Collection of specimens and laboratory methods

At all study visits, patients underwent a physical examination and completed an interview with questions concerning demographic characteristics and sexual behaviors. A routine medical history was taken by a clinician who also performed external genital and bimanual pelvic examinations as previously described [22]. Cervicovaginal lavage (CVL) specimens for detection of HIV were collected as follows. With a speculum in place, a lavage of the cervix and vagina was performed using a plastic mixing cannula connected to a 10-ml syringe. Five milliliters of sterile physiologic saline was directed towards the cervix and the washings were aspirated (noting the volume aspirated) from the posterior vaginal fornix using the same syringe and mixing cannula. The sample was tested for occult blood using a Hemastix test stick, and if positive, was not used for purposes of HIV detection. Fifteen milliliters of peripheral blood was collected into tubes containing EDTA [or acid-citrate-dextrose (ACD)] and analyzed using the FACSCCount analyzer (Becton Dickinson Biosciences, San Jose, California, USA) to determine the number of CD4, CD8 and CD3 cells/ μ l of blood and for HIV-1 and HIV-2 qualitative and quantitative RNA and DNA assays. Quantitative and qualitative assays for HIV-1 and HIV-2 plasma and cervicovaginal RNA, and peripheral blood mononuclear cell (PBMC) DNA and cervicovaginal DNA were performed using PCR-based assays developed at Roche Molecular Systems (Pleasanton, California, USA) [23] as previously described [15,21]. Cervical infections with gonorrhea and chlamydia were assessed by PCR. Syphilis serology was performed using the rapid plasma reagin (RPR) test followed by a *Treponema pallidum*-specific hemagglutination assay (TPHA). *Trichomonas vaginalis*, *Candida* sp. and the presence of clue cells were identified by wet-mount microscopy of vaginal smears.

Statistical methods

For both the HIV-1 and HIV-2 RNA or DNA assays, samples, which were positive by the more sensitive qualitative test but were negative by the less sensitive quantitative test were categorized as 'positive, below level of quantification'. In univariate analyses, Pearson χ^2 or Fisher's exact tests were performed to compare demographic and laboratory characteristics with respect to HIV-1 versus HIV-2 infection and to compare these factors with detection of vaginal HIV RNA among women with HIV-1 or HIV-2. Mantel-Haenszel trend tests were also used to compare ordered categorical variables. Continuous variables (e.g., CD4 cell count and $\log_{10}$ plasma RNA) were assessed using the Wilcoxon rank-sum test or Student's *t*-test, when medians or means were compared, respectively. Univariable and multivariable analysis of initial visit detection of vaginal HIV RNA was conducted with logistic regression with the multivariable analysis simultaneously adjusting for all factors in the model. With regards to the relationship between levels of vaginal HIV RNA and plasma HIV RNA, correlation coefficients and linear regression techniques were utilized.

In the 6-week study, data from multiple study visits per patient were incorporated into a single analysis to assess relationships between the dependent variable (cervicovaginal RNA detection), and independent variables, including HIV type, $\log_{10}$ plasma HIV RNA, CD4 cell count, timing of menses, and sexual intercourse using generalized estimating equations (GEEs) [24]. In this analysis, we assumed an exchangeable working correlation structure to

account for intra-subject correlation. Data analyses were conducted using SAS 9.1 for Windows (SAS Institute, Cary, North Carolina, USA).

Results

Characteristics of the study population

At baseline, women with HIV-1 ($n=168$) as compared with HIV-2 ($n=50$) were younger (mean age 31.3 versus 35.3 years; $P=0.002$), more likely to be widowed ($P=0.01$), and had delivered fewer children ($P<0.001$), but were similar with regard to use of birth control, cigarette smoking, vaginal douching, and prior employment as a commercial sex worker (Table 1). Women with HIV-1 were similar to those with HIV-2 with respect to prevalence of most sexually transmitted infections, with the exception of vaginal candidiasis ($P=0.05$), which was more common in women with HIV-1 as compared with women with HIV-2. Women with HIV-1 and HIV-2 had similar abnormal genital examination findings, although women with HIV-1 were more likely to have abnormal vaginal discharge noted ($P=0.04$).

Not surprisingly, women infected with HIV-1 had significantly higher $\log_{10}$ plasma HIV RNA levels (mean 4.9 versus 2.4 copies/ml; $P<0.001$). However, we observed similar mean CD4 cell counts (301 versus 344 cells/ μ l; $P=0.3$) and $\log_{10}$ PBMC HIV DNA levels (mean 1.6 versus 1.6 copies/mg; $P=0.9$) in women infected with HIV-1 as compared with HIV-2. None of the patients were receiving ARTs.

Cross-sectional analysis of presence and level of HIV RNA and DNA in the genital tract

In the cross-sectional study, women with HIV-1 were significantly more likely to have detectable HIV RNA detected in CVL specimens than were women with HIV-2 (78 versus 58%, respectively; $P=0.005$; Table 2). In women with detectable HIV RNA, the levels of HIV RNA was also significantly higher in women with HIV-1 as compared with HIV-2 ($P=0.005$), with 17% of those with HIV-1, as compared with none of those with HIV-2, having levels above 10 000 copies of RNA per milliliter. Detection of HIV DNA was marginally more common in women with HIV-1 as compared with HIV-2 (50 versus 33%, $P=0.06$), although in those with detectable HIV DNA, levels were similar in HIV-1 as compared with HIV-2 ($P=0.4$).

Logistic regression was used to assess predictors of detection of cervicovaginal HIV RNA. In separate univariate analyses, low CD4 cell counts and increased plasma HIV RNA were significantly and similarly associated with detection of cervicovaginal HIV-1 or HIV-2 RNA (Table 3). Detection of HIV RNA in CVL specimens was significantly associated with high (quantifiable) levels as compared with undetectable levels of HIV-1, but not HIV-2 DNA; however, risk estimates were similar in HIV-1 and HIV-2. Cervicovaginal HIV-1 or HIV-2 RNA was always detected in the women with genital/cervical ulcers ($n=17$) and cervical discharge ($n=5$, data not shown). Detection of HIV RNA was not significantly associated with measured demographic characteristics or other examination findings, although we observed nonsignificant trends of increased vaginal HIV-1 and HIV-2 RNA detection associated with increased age, vaginal candida, elevated vaginal pH, and abnormal cervical examination findings, including bleeding (data not shown).

We then combined data from the HIV-1 and HIV-2-infected women in order to assess the independent associations of HIV type, immune suppression (CD4 cell counts), HIV plasma RNA, and local HIV DNA detection (Table 3) with cervicovaginal HIV detection. In multivariable logistic regression analysis, detection of HIV RNA remained significantly associated with increased plasma HIV RNA levels [odds ratio (OR) =1.9 per $\log_{10}$ increase in plasma HIV RNA; $P=0.006$]. Detection of cervicovaginal RNA was marginally associated with quantifiable cervicovaginal HIV DNA levels [OR =5.4, 95% confidence

interval (CI) =0.6–45.9; $P=0.11$ compared with undetectable HIV DNA]. There were trends for HIV-2 infection and low CD4 cell counts associated with HIV RNA detection, but these did not attain statistical significance.

Increased plasma HIV RNA level was also correlated with increased level of HIV RNA detected in CVL (Fig. 1), both in HIV-1 ($r^2=0.20$) and HIV-2 ($r^2=0.29$) infection. In separate linear regression analyses (data not shown), each $\log_{10}$ increase in HIV-1 plasma HIV-1 RNA was associated with a 0.59 $\log_{10}$ increase in HIV-1 RNA level in CVL ($P<0.001$) although in women with HIV-2, each $\log_{10}$ increase in HIV-2 plasma HIV-1 RNA was associated with a 0.38 $\log_{10}$ increase in HIV-2 RNA level in CVL ($P<0.001$).

Logistic regression was used to assess predictors of detection of vaginal HIV DNA (Table 4). Similar to what was seen with respect to HIV RNA detection, decreased CD4 cell counts were associated with DNA detection, although these did not reach statistical significance in either HIV-1 or HIV-2 infection. Increased plasma HIV RNA was significantly associated with detection of HIV DNA in CVL in each infection. When data from HIV-1 and HIV-2-infected women were combined in multivariable analyses, HIV plasma RNA level remained significantly associated with detection of HIV DNA in CVL samples (OR =2.0 per $\log_{10}$ increase in HIV plasma RNA). However, after adjusting for HIV plasma viral load, neither HIV type (OR =2.0, 95% CI 0.6–6.7 for HIV-2 compared with HIV-1) nor CD4 cell level were associated with HIV DNA detection in CVL.

Longitudinal detection of cervicovaginal HIV RNA

In order to better understand the patterns of vaginal HIV shedding in HIV-1 or HIV-2-infected women, we undertook a longitudinal study, sampling 31 patients with CVL every 3 days for up to 6 weeks. In total, 265 visits were completed at which cervicovaginal HIV shedding was assessed, for a mean of 8.5 visits per woman. During follow-up, HIV RNA was detected in at least one visit in 18 (90%) of 20 HIV-1-infected women and nine (82%) of 11 HIV-2-infected women (Fig. 2). Approximately half (45%) of the closely followed women had intermittently detected (in 20–80% of visits) HIV-1 or HIV-2 RNA, although 42% of women had usual or universal detection (in >80% of visits) of HIV-1 or HIV-2 RNA. There was no difference in the percentage of visits that women detected HIV RNA between HIV-1 and HIV-2-infected women ($P=0.9$).

Logistic regression was used to assess predictors of detection of cervicovaginal HIV RNA in these closely followed women. In separate univariate analyses, decreased CD4 cell counts were significantly associated with increased CVL HIV RNA detection in HIV-2 infection but this was not significant within HIV-1 infection. Increased plasma HIV RNA levels (OR =1.8 per $\log_{10}$ copies/ml, $P=0.08$ in HIV-1; OR =1.6 per $\log_{10}$ copies/ml, $P=0.03$ in HIV-2) were similarly associated with detection of cervicovaginal HIV-1 or HIV-2 RNA (Table 5). Lastly, detection of cervicovaginal HIV RNA was increased with recent menses in women with HIV-1 or HIV-2. When data from both HIV-1 and HIV-2-infected women were combined in multivariable analyses to assess the independent associations of HIV type, immune suppression (CD4 cell counts), HIV plasma RNA, menses, and recent sexual intercourse with cervicovaginal HIV detection, detection of HIV RNA remained significantly associated with low CD4 cell levels below 200 cells/ μ l (OR =6.3, 95% CI =1.2–32.8) and menses in the past 8 days compared with menses 22–31 days ago (OR =2.5, 95% CI =1.5–4.1).

Discussion

The present study is the first to compare both the presence and levels of HIV RNA and HIV DNA in the genital tract among women with HIV-1 to those with HIV-2 infection. As this

study was conducted prior to the availability of HAART in Senegal, none of the women were receiving ARTs. We assessed the proportion of CVL samples in which HIV-1 or HIV-2 RNA and DNA was detected both at the initial study visit and during follow-up using intensive sampling. Using this comprehensive approach, we found CVL HIV RNA in initial visit samples from 78 and 58% of HIV-1 and HIV-2-infected women, respectively, and at some visit in 95 and 82%, respectively, of women followed longitudinally. These proportions are higher, particularly among women with HIV-1, than the 41 and 33% found by Seck *et al.* [12] in a similar population. We found CVL HIV DNA in initial visit samples from 50 and 33% of HIV-1 and HIV-2-infected women, respectively. Again, these proportions are significantly higher than those found by Ghys *et al.* [11] (24 and 5%, respectively); however, the women included in that study were commercial sex workers who were observed to have lower vaginal HIV detection rates than other HIV-infected women in the study by Seck *et al.* [12]. Our observed greater detection rate of CVL HIV RNA and DNA may also reflect greater sensitivity of our PCR assays. We detected cervicovaginal HIV RNA in the vast majority of visits (80%) over a 6-week period among 50% of HIV-1 and 45% of HIV-2-infected women. Conversely, cervicovaginal HIV RNA was never detected in only 5 and 18% of HIV-1 and HIV-2-infected women, respectively. In a study of 55 HIV-1-infected women followed over two menstrual cycles, Coombs *et al.* [25] found genital HIV RNA was detected continuously in 29% and never detected in 13%. Thus, the lack of detection of HIV RNA in genital secretions in a single sample does not indicate that a woman will never shed HIV, as few untreated HIV-infected women, regardless of HIV type, appear to have consistently negative results over time.

Although we observed that decreased CD4 cell count and higher levels of plasma HIV RNA were each independently associated, in multivariable analyses, with detection of cervicovaginal HIV RNA, we found higher plasma HIV RNA levels, but not CD4 cell count, to be independently associated with increased levels of cervicovaginal HIV RNA, as well as detection of cervicovaginal HIV DNA. Similarly, most [6,12,25,26], but not all [11,18] studies which have evaluated both HIV plasma RNA levels and CD4 cell count, found HIV plasma load, but not CD4 cell count, to be independently associated with detection of HIV in the genital tract, suggesting that HIV plasma load, rather than CD4 level of immune suppression, may be the more important determinant of vaginal HIV shedding.

Our finding of no difference in the risk of detecting HIV RNA or DNA in the genital tract between HIV-1 and HIV-2-infected women with CD4 cell counts below 200 cells/ μ l is most likely due to the fact that these women, regardless of HIV type, have high levels of HIV plasma RNA. However, the small sample size in women with HIV-2, especially in those with low CD4 cell counts, limited our ability to observe such differences. In contrast, among women with higher CD4 cell counts (>200 cells/ μ l), HIV-2 plasma loads are generally much lower than HIV-1 plasma loads.

Although detection of cervicovaginal HIV DNA and RNA was associated with higher plasma HIV RNA levels among both HIV-1 and HIV-2-infected women, we also found that among women with low-plasma HIV RNA levels (<5000 copies/ml), 45 and 31% of women infected with HIV-1 and HIV-2, respectively, had HIV RNA detected in vaginal samples, and 26 and 19%, respectively, had HIV DNA detected in vaginal samples, suggesting a finite risk of infectivity in untreated women even when plasma viral loads are low. Even among HIV-infected women being treated with HAART in developed countries, approximately 15% of women receiving HAART and 3% with undetectable plasma viral loads demonstrate detectable levels of HIV RNA in vaginal samples [27], and in Kenya 35% of women initiating HAART had detectable genital HIV-1 RNA in the cervix or vagina 1 month after treatment [28]. These findings may be partly explained by local HIV production [29].

Although several studies have evaluated determinants of cervicovaginal shedding of HIV among HIV-1-infected women, only one previous study [12] has assessed determinants of genital tract shedding among HIV-2-infected women or compared predictors of HIV-2 as compared with HIV-1 detected in vaginal secretions. In that previous study, Seck *et al.* [12] found vaginal HIV-2 RNA shedding to be associated with low CD4 cell count; HIV-2 plasma RNA levels were not assessed. Although our study was limited by the small number of patients with HIV-2 infection ($n=50$), but it is the first to assess levels of HIV-1 and HIV-2 shedding, and also to longitudinally assess shedding over time.

In our study, the most important correlates of both detection and levels of cervicovaginal HIV RNA (HIV-1 and HIV-2) were higher plasma HIV RNA levels and secondarily, elevated vaginal pH; once these factors were taken into account, HIV type (HIV-1 versus HIV-2) was no longer associated with vaginal HIV RNA. Lower (more acidic) vaginal pH may lead to partial inactivation of HIV, and hence, lower vaginal HIV levels [30]. Elevated vaginal pH also may reflect a diagnosis of bacterial vaginosis; several pathogens associated with bacterial vaginosis appear to be associated with HIV-inducing factor [31]. We noted increased HIV shedding in the week after menstruation and in women without regular menstruation. However, consensus regarding the relationship between day of cycle and cervicovaginal HIV shedding is lacking [25,32–35].

In summary, we found significantly lower rates and levels of HIV-2 RNA in the female genital tract than HIV-1 RNA and shedding strongly correlated with plasma viral load irrespective of virus type. After taking into account plasma HIV RNA load, neither HIV type nor any local factors were independently associated with detection of vaginal HIV RNA. Among women in whom HIV RNA was detected, the only factor independently associated with increased levels of cervicovaginal HIV RNA was HIV-2 infection, even after adjustment for plasma HIV load. This increased level of HIV RNA may reflect an unmeasured effect of a longer period of immunosuppression among HIV-2-infected women as compared with HIV-1-infected women with similar plasma loads and CD4 cell counts [15]. Finally, in untreated women with HIV-1 or HIV-2 infection, over 80% of closely monitored women, regardless of HIV type, had at least intermittent HIV RNA detection during every 3-day sampling over a 6-week time period. These data may help explain the different transmission rates between HIV-1 and HIV-2 and may provide new insights regarding prevention.

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Fig. 1. HIV-1 and HIV-2 CVL and plasma RNA viral loads.
br>CVL, cervicovaginal lavage.

Fig. 2. Distribution of CVL HIV RNA detection in closely followed women ($n = 1$ women, $n = 265$ samples)
CVL, cervicovaginal lavage.

Table 1

Characteristics of women infected with HIV-1 or HIV-2.

Characteristic	HIV serologic status	
	HIV-1 (n =168)	HIV-2 (n =50)
<i>Age (mean years $\pm$ SD)</i>	<i>31.3 $\pm$ 7.8</i>	<i>35.3 $\pm$ 7.4</i>
<i>Marital status</i>		
Married	78/165 (47%)	34/50 (68%)
Widowed	44/165 (27%)	5/50 (10%)
Divorced	30/165 (18%)	7/50 (14%)
Never married	13/165 (8%)	4/50 (8%)
<i>Parity (median, inter-quartile range)</i>	<i>3 (1–5)</i>	<i>5 (3–7)</i>
Birth control	25/166 (15%)	10/49 (20%)
Tobacco use	13/168 (8%)	3/50 (6%)
History of commercial sex work	18/168 (11%)	4/50 (8%)
Vaginal douching	33/93 (35%)	13/35 (37%)
<i>STIs/wet-mount findings</i>		
Gonorrhea	2/126 (2%)	0/40 (0%)
Chlamydia	2/132 (2%)	1/38 (3%)
Syphilis	10/139 (7%)	2/44 (5%)
Trichomoniasis	7/123 (6%)	2/39 (5%)
<i>Vaginal candidiasis</i>	<i>43/123 (35%)</i>	<i>7/39 (18%)</i>
Vaginal pH >5.0	74/150 (49%)	28/45 (62%)
Clue cells	40/123 (33%)	11/39 (28%)
<i>Examination findings</i>		
Abnormal cervical examination	146/159 (92%)	43/47 (92%)
Cervical bleeding	48/145 (33%)	18/44 (41%)
Cervical discharge	4/160 (3%)	1/46 (2%)
Cervical ulcer	12/159 (8%)	3/47 (6%)
<i>Abnormal vaginal discharge</i>	<i>113/159 (71%)</i>	<i>26/47 (55%)</i>
Genital ulcer	15/160 (9%)	2/47 (4%)
CD4 cell count (mean cells/ μ l $\pm$ SD)	301 $\pm$ 252	344 $\pm$ 231
<i>Log₁₀ plasma HIV RNA (mean copies/ml $\pm$ SD)</i>	<i>4.9 $\pm$ 1.2</i>	<i>2.4 $\pm$ 1.9</i>
Log ₁₀ PBMC HIV DNA (mean copies/ μ g $\pm$ SD)	1.6 $\pm$ 0.8	1.6 $\pm$ 1.0

Note: Factors significantly different ($P < 0.05$) in those with HIV-1 as compared with HIV-2 are denoted with italics. PBMC, peripheral blood mononuclear cell; STI, sexually transmitted infections.

Table 2

Cross-sectional detection of HIV RNA and DNA in cervicovaginal lavage specimens.

HIV RNA and DNA in CVL samples	HIV serologic status		
	HIV-1 (<i>n</i> =168)	HIV-2 (<i>n</i> =50)	<i>P</i>
Detection of HIV RNA	131 (78%)	29 (58%)	0.005
Quantity among those with HIV RNA detected ^a			0.005 ^b
Below level of quantification	21/121 (17%)	8/24 (33%)	
10–999 copies/ml	52/121 (43%)	13/24 (54%)	
1000–9999 copies/ml	28/121 (23%)	3/24 (13%)	
At least 10 000 copies/ml	20/121 (17%)	0/24 (0%)	
Detection of HIV DNA	60/121 (50%)	13/40 (33%)	0.06
Quantity among those with HIV DNA detected ^c			0.4 ^a
Below level of quantification	22/43 (51%)	4/9 (44%)	
1–9 copies/μg	7/43 (16%)	1/9 (11%)	
10–99 copies/μg	11/43 (26%)	2/9 (22%)	
At least 100 copies/μg	3/43 (7%)	2/9 (22%)	

CVL, cervicovaginal lavage.

^a 10 HIV-1 and five HIV-2 samples were qualitative test positive but did not have the quantification assay performed.^b *P* calculated for samples in which RNA or DNA was detected.^c Seventeen HIV-1 and four HIV-2 samples were qualitative test positive but did not have the quantification assay performed.

Table 3

Correlates of cervicovaginal lavage HIV RNA detection in cross-sectional study.

	Univariate OR (95% CI), by HIV type		^a Adjusted OR (95% CI) HIV RNA
	HIV-1 RNA	HIV-2 RNA	
HIV type			
HIV-1	NA	NA	(ref)
HIV-2	NA	NA	2.5 (0.6–10.2)
CD4 cell count (cells/ μ l)			
Above 500	1.0 (ref)	1.0 (ref)	1.0 (ref)
200–500	1.2 (0.5–3.1)	3.0 (0.6–14.9)	0.9 (0.3–2.8)
Below 200	4.3 (1.4–12.8)	21.0 (2.9–151.4)	2.2 (0.6–8.0)
Log ₁₀ plasma HIV RNA	2.7 (1.8–4.2)	2.1 (1.4–3.3)	1.9 (1.3–2.8)
CVL DNA			
Negative (qual negative)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Low (qual positive, quant = 0)	0.8 (0.3–2.2)	0.9 (0.1–7.6)	0.6 (0.2–1.9)
High (quant 1)	9.1 (1.1–72.4)	3.7 (0.4–37.7)	5.4 (0.6–45.9)

CI, confidence interval; CVL, cervicovaginal lavage; OR, odds ratio.

^aOR from multivariable logistic regression, adjusting for all factors in the table.

Table 4

Correlates of cervicovaginal lavage HIV DNA detection in cross-sectional study.

	Univariate OR (95% CI) by HIV type		*Adjusted OR (95% CI) HIV DNA
	HIV-1 DNA	HIV-2 DNA	
HIV type			
HIV-1	NA	NA	(ref)
HIV-2	NA	NA	2.0 (0.6–6.7)
CD4 cell count (cells/ μ l)			
Above 500	1.0 (ref)	1.0 (ref)	1.0 (ref)
200–500	1.5 (0.5–4.3)	0.3 (0.0–4.3)	0.7 (0.2–2.0)
Below 200	2.1 (0.7–6.0)	4.7 (0.7–31.0)	0.9 (0.3–2.9)
Log ₁₀ plasma HIV RNA	1.9 (1.3–2.8)	2.9 (1.3–6.1)	2.0 (1.3–2.9)

CI, confidence interval; OR, odds ratio.

Table 5Correlates of cervicovaginal lavage HIV RNA detection in closely followed women (n = samples analyzed).

	Univariate OR (95% CI), by HIV type		^a Adjusted OR (95% CI) HIV RNA
	HIV-1 RNA (n =160)	HIV-2 RNA (n =105)	
HIV type			
HIV-1	NA	NA	1.0 (ref)
HIV-2	NA	NA	1.1 (0.2–7.2)
CD4 cell count (cells/ μ l)			
Above 500	1.0 (ref)	1.0 (ref)	1.0 (ref)
200–500	1.8 (0.4–8.2)	6.9 (0.6–85.7)	2.9 (0.6–15.1)
Below 200	3.3 (0.6–20.2)	26.2 (4.2–163.5)	6.3 (1.2–32.8)
Log ₁₀ plasma HIV RNA	1.8 (0.9–3.5)	1.6 (1.1–2.5)	1.1 (0.7–1.8)
Menses			
22–31 days ago	1.0 (ref)	1.0 (ref)	1.0 (ref)
16–21 days ago	1.0 (0.4–2.5)	1.0 (0.6–1.5)	1.1 (0.5–2.2)
9–15 days ago	1.5 (0.6–3.5)	0.9 (0.5–1.7)	1.3 (0.6–2.8)
0–8 days ago	2.5 (1.3–4.8)	1.8 (0.9–3.5)	2.5 (1.5–4.1)
Never had menses	2.6 (0.6–12.0)	1.4 (0.2–9.8)	2.7 (0.8–8.9)
Sex in the past 2 days	0.9 (0.5–1.8)	1.5 (0.3–7.3)	1.1 (0.4–2.7)

CI, confidence interval; OR, odds ratio.

^aOR from multivariable logistic regression, using generalized estimating equations, adjusting for all factors in the table.