

Cervicovaginal HIV-1 Shedding in Women Taking Antiretroviral Therapy in Burkina Faso: A Longitudinal Study

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Background: Antiretroviral therapy (ART) reduces transmission of HIV-1. However, genital HIV-1 can be detected in patients on ART. We analyzed factors associated with genital HIV-1 shedding among high-risk women on ART in Burkina Faso.

Methods: Plasma viral load (PVL) and enriched cervicovaginal lavage HIV-1 RNA were measured every 3–6 months for up to 8 years. Random-effects logistic and linear regression models were used to analyze associations of frequency and quantity of genital HIV-1 RNA with behavioral and biological factors, adjusting for within-woman correlation. The lower limit of detection of HIV-1 RNA in plasma and eCVL samples was 300 copies per milliliter.

Results: One hundred and eighty-eight participants initiated ART from 2004 to 2011. PVL was detectable in 16% (171/1050) of visits, in 52% (90/174) of women. Cervicovaginal HIV-1 RNA was detectable in 16% (128/798) of visits with undetectable plasma HIV-1 RNA in 45% (77/170) of women. After adjusting for PVL, detectable cervicovaginal HIV-1 RNA was independently associated with abnormal vaginal discharge and use of nevirapine or zidovudine

vs. efavirenz and stavudine, respectively; longer time on ART and hormonal contraception were not associated with increased shedding. The presence of bacterial vaginosis, herpes simplex virus-2 DNA, and the use of nevirapine vs efavirenz were independently associated with an increased quantity of cervicovaginal HIV-1 RNA.

Conclusions: Certain ART regimens, abnormal vaginal discharge, bacterial vaginosis, and genital herpes simplex virus-2 are associated with HIV-1 cervicovaginal shedding or quantity in women on ART after adjusting for PVL. This may reduce the effectiveness of ART as prevention in high-risk populations.

Key Words: antiretrovirals, HIV-1 RNA, cervicovaginal lavage, nevirapine, bacterial vaginosis, herpes simplex virus type-2

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INTRODUCTION

Plasma viral load (PVL) is a key determinant of sexual HIV-1 transmission,^{1–4} and transmission rarely occurs if PVL is below 400 copies per milliliter.^{5,6} A large multicentre randomized controlled trial confirmed a pronounced impact of antiretroviral therapy (ART) on the risk of sexual transmission of HIV-1 in serodiscordant couples, previously seen in cohort studies.^{7–9}

Most transmission occurs in the context of exposure to genital secretions, and it is probable that the concentration of HIV-1 virions in the genital tract contributes to transmission risk. The infectiousness of the cell-free and cell-associated HIV-1 components of genital secretions is little understood, although an association with genital viral load, independent of PVL, has been shown for vertical transmission.¹⁰ The independent impact of genital viral load on heterosexual transmission was recently confirmed in a large trial of acyclovir for preventing HIV-1 transmission in serodiscordant couples in Africa (the Partners in Prevention Trial, PiP).⁵ In a secondary analysis, the trial demonstrated that the risk of female-to-male sexual transmission increased 1.67-fold with each 1.0 log₁₀ copies per milliliter increase in endocervical swab HIV-1 RNA concentration.

Determinants of HIV-1 genital shedding include PVL^{11,12} and both asymptomatic and symptomatic reproductive tract infections (RTIs) in ART-naïve patients.^{13,14} Although genital HIV-1 RNA has been shown to rapidly decline after the initiation of ART,^{15,16} it can be present in semen and cervicovaginal secretions in patients on stable

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The Members of Yereon Study Group are listed in the Appendix 1.

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ART,^{11,17–20} and poor adherence can be predictive of cervical and vaginal shedding.¹⁷ Few studies have been conducted in areas of high RTI prevalence, limiting the power to detect other predictors of shedding.¹⁹ In analyses of seminal HIV-1 RNA, concurrent sexually transmitted infections (STIs) and genital inflammation have been shown to interfere with the suppressive effect of ART.²⁰ In conjunction with the resurgence of high-risk sexual behavior, the rise in new cases of STIs in men who have sex with men on ART has been proposed as a cause of the persistently high incidence of HIV-1 in some high-income settings.^{18,21,22}

The relevance of randomized controlled trials of treatment as prevention in serodiscordant couples to other high-risk groups where adherence to ART might be lower, and the durability of this protective effect are unclear.^{8,9,23} It therefore remains important to measure genital shedding of cervicovaginal HIV-1 RNA among high-risk populations on ART to estimate the potential impact on transmission. Female sex workers (FSWs) represent epidemiologically important core groups, in whom a higher prevalence of RTIs and genital inflammation might also limit the effect of ART as prevention. In this study, we analyze the frequency, quantity, and determinants of HIV-1 genital shedding in a cohort of FSWs taking ART over 8 years in Burkina Faso.

METHODS

Participants and Study Procedures

Study participants were HIV-1-seropositive FSWs living in Bobo-Dioulasso, Burkina Faso, who initiated ART between August 2004 to February 2011. Participants were derived from an open cohort of 917 high-risk women established in 1998, which underwent 3 further phases of recruitment in 2003, 2007, and 2009.^{24–26} Women working in the streets and bars of Bobo-Dioulasso were eligible for the cohort if they reported at least 1 transactional sex act per week, were aged 16 years or older, and were willing to undergo regular testing for HIV and STIs. Women were recruited from local organizations for people living with HIV/AIDS, using the same criteria. Since 2004, HIV-infected participants in Burkina Faso who meet the WHO eligibility criteria for ART initiation have had access to ART.²⁷ Women with HIV clinical stages III or IV or with a CD4+ count ≤ 200 cells/ μ L (≤ 350 cells/ μ L from 2009) have been treated with standard ART. First-line treatment is primarily nonnucleoside reverse transcriptase inhibitor-based as follows: women on adequate contraception receive efavirenz, whereas those not using contraception receive nevirapine.²⁶ Women desiring pregnancy are switched to nevirapine. Protease inhibitors (PI) (indinavir or nelfinavir with ritonavir) are mainly used as second-line treatment. Women already on ART at the enrollment visit were originally provided with medications at the Bobo-Dioulasso University Teaching Hospital.

The research protocol was approved by the institutional review board at Centre Muraz and the research ethics committees at the Burkina Faso Ministry of Health and the London School of Hygiene and Tropical Medicine. All women provided written informed consent.

Participants were followed approximately every 3–6 months. At enrollment, an interviewer administered a ques-

tionnaire eliciting sociodemographic and behavioral characteristics and sexual health information. At subsequent visits, behavioral, sexual health, and treatment information from the intervening period was collected. A clinician performed a gynecological exam and collected genital samples at each visit. An abnormal cervix was defined as friable, inflamed, or with lesions on exam, and abnormal vaginal discharge was defined as copious, malodorous, or of abnormal consistency. The visit was deferred during menses until 2 days after cessation of bleeding.

ART adherence counseling used an intensive method whereby women on ART were seen monthly by clinical psychologists to address any issues, and group sessions were convened by local “peer educators”, as previously described.²⁸ Adherence was measured by self-report and pill count at each visit. Treatment failure was defined virologically as detectable plasma viremia (>300 copies/mL) on 2 consecutive measures after 6 months of treatment or after viral suppression.²⁶ Participants were regularly tested for STIs and treated according to national guidelines.

Laboratory Investigations

At each scheduled visit, an enriched cervicovaginal lavage (eCVL) was performed by infusing 2 mL of normal saline into the vagina for 60 seconds and collecting it into a cryotube. A swab was then rotated 360 degrees in the cervical os and expressed into the cryotube.²⁹ Vaginal smears were examined using wet-mount microscopy to detect *Trichomonas vaginalis* and *Candida albicans* hyphae and spores. Heat-fixed vaginal smears were Gram stained and Nugent scored to diagnose bacterial vaginosis (BV).³⁰ Diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* was done post hoc using polymerase chain reaction (PCR) (Amplicor *Chlamydia trachomatis/Neisseria gonorrhoeae* PCR assay; Roche, Rotkreuz, Switzerland) for cervical swabs dating from 2007 onwards due to concerns about DNA degradation in older samples although in storage. Since 2003, herpes simplex virus type 2 (HSV-2) serology was done at each phase enrollment visit, using Kalon IgG2-enzyme-linked immunosorbent assay test (Kalon Biologicals, Surrey, United Kingdom).

HIV serology was tested using a Determine-1/2 rapid kit (Abbott, Tokyo, Japan) with Genie-II (Bio-Rad, Paris, France) confirmation. CD4+ counts were determined using FACScan (Becton Dickinson) at each visit. Plasma was collected every 6–12 months for HIV-1 RNA measurement. Plasma and eCVL HIV-1 RNA was quantified using real-time PCR (Generic HIV viral load; Biocentric, Bandol, France), validated for use with the predominant HIV-1 subtypes found in Burkina Faso.³¹

For HSV-2-seropositive women, HSV-2 DNA was extracted from 200 μ L of eCVL fluid using the QIAamp DNA minikit (Qiagen, West Sussex, United Kingdom) and eluted in 100- μ L buffer. HSV-2 DNA was amplified from 5 μ L of eluate by TaqMan real-time PCR using the ABI Prism 7000 Sequence Detection Systems, as previously described, and quantified using the HSV-2 Quantitated External Control (Tebu-Bio, Le-Perray-en-Yvelines, France).³² The lower limit

of detection was 300 copies per milliliter for HIV-1 RNA and HSV-2 DNA.

To detect contamination of eCVL samples with semen, the detection of sperm was done on extracted DNA, using primers SRY-F (5'-CGC ATT CAT CGT GTG GTC TCG-3') and SRY-R (5'-ATT CTT CGG CAG CAT CTT CGC-3') to detect the short-tandem repeat (STR) on the Y chromosome, using a single qualitative PCR.³³ Visual examination of eCVL samples was done by the clinician and independently by a laboratory technician, and the presence of blood was documented.

Statistical Analyses

Statistical analyses were performed using Stata version 12.0 (StataCorp, College Station, TX). Baseline visits are considered the last visit before ART initiation or at study enrollment if the participant was already on ART. Time on ART was calculated from the date of ART initiation even if it preceded study enrollment. Quantities of plasma and cervicovaginal HIV-1 RNA and cervicovaginal HSV-2 DNA were transformed to log₁₀ copies per milliliter. To determine factors associated with detectable shedding, odds ratios (ORs) were estimated using logistic regression for all visits on ART, adjusting for within-woman correlation using random effects with an independent covariance structure. Based on literature demonstrating the importance of PVL in shedding, estimates were also adjusted for PVL (as a categorical variable: undetectable, 2.48–3.9, 4.0–4.9, and ≥5.0 log₁₀ copies/mL). The *P* values were obtained using likelihood ratio tests. A multivariable logistic regression model was constructed using a conceptual hierarchical framework including risk factors either decided a priori, namely PVL and adherence as categorical values, or independently associated with the presence of eCVL HIV-1 RNA after adjusting for PVL using *P* ≤ 0.10 as the inclusion criterion.

The correlation between plasma and cervicovaginal HIV-1 RNA quantities in samples with detectable HIV-1 RNA was assessed using the Spearman's rank correlation coefficient. Random-effects linear regression, restricted to those visits with detectable cervicovaginal HIV-1 RNA, was used to assess factors associated with the quantity of cervicovaginal HIV-1 RNA and was also conducted adjusting for PVL as a categorical value. The *P*-values were calculated using the Wald test. A multiple linear regression model was constructed in the same fashion as the logistic model.

RESULTS

Participant Characteristics

Over the 8-year period, 199 women in the cohort were eligible for the study: 175 who initiated ART during cohort follow-up and 24 who were already receiving ART at enrollment for a median of 265 days [interquartile range (IQR): 75–379]. Data on HIV-1 RNA in eCVL samples were available for 188 women (Table 1). There was no difference in clinical or sociodemographic factors for those without samples. The median age at enrollment was 32 years; 45% had received no formal education; and 11% percent defined them-

selves as professional sex workers, defined as those who use transactional sex as their primary source of income.

The majority of participants (90%) were HSV-2 seropositive at baseline, and of those with available data, 15% (16/105) had detectable cervicovaginal HSV-2 DNA during their pre-ART visit. The most common RTI at baseline was BV present in 37% of women, and 13% had ulcers or vesicles on exam.

At the pre-ART initiation visit, the median CD4+ count was 177 cells/μL (IQR: 122–228), and the mean PVL was 4.95 log₁₀ copies per milliliter (SD: ±0.84). Most women (78%) had detectable eCVL HIV-1 RNA, and the mean viral load was 3.95 log₁₀ copies per milliliter (SD: ±0.79) in those with detectable eCVL HIV-1 RNA. The most common initial ART regimens were efavirenz, zidovudine, and lamivudine (taken by 37% of women), and nevirapine, stavudine, and lamivudine (taken by 27%). Only 2% initiated a PI-containing regimen.

TABLE 1. Demographic and Clinical Characteristics of Cohort participants Receiving Antiretroviral Therapy in Bobo-Dioulasso, Burkina Faso (N = 188)

Characteristic at Baseline Visit*	Value† (n = 188)
Age—yrs (range)	32 (18–52)
Number who have not received any education (%)	84 (45)
Full-time sex workers (%)	21 (11)
Duration of sex work—yrs (IQR)	11 (7–20)
Median number of sex acts in the past week (range)	4.4 (0–70)
Current hormonal contraceptive use (%)	26 (14)
Practice regular vaginal washing (%)	152 (81)
HSV-2 seropositive (%)	168/186 (90)
Number with detectable cervicovaginal HSV-2 DNA (%)	16/105 (15)
RTI	
Bacterial vaginosis (%)	70 (37)
<i>C. albicans</i> (%)	18 (10)
<i>T. vaginalis</i> (%)	6 (3)
Ulcers or vesicles on exam (%)	24 (13)
Number receiving ART at study enrollment (%)	24 (13)
Number with pre-ART CD4+ count <200 cells/μL (%)	111/163 (68)
Median CD4+ count (IQR)	177 (122–228)
Number with detectable pre-ART plasma HIV-1 RNA (%)	155/158 (98)
Mean plasma HIV-1 RNA, log ₁₀ copies/mL (±SD)	4.95 (±0.84)
Number with detectable pre-ART cervicovaginal HIV-1 RNA (%)	113/144 (78)
Mean cervicovaginal HIV-1 RNA viral load among shedders, log ₁₀ copies/mL (±SD)	3.95 (±0.79)
First-line ART regimen (%)	
Efavirenz/zidovudine/lamivudine	70 (37)
Efavirenz/stavudine/lamivudine	21 (11)
Nevirapine/zidovudine/lamivudine	42 (22)
Nevirapine/stavudine/lamivudine	51 (27)
Protease inhibitor or other	4 (3)

*Baseline visit is considered the last visit before ART initiation or at study enrollment if already on ART.

†Data are number of participants (%), unless otherwise noted. Some denominators vary due to missing data. There are no pre-ART data for the 24 women who were taking ART before cohort enrollment.

The median follow-up time on ART was 3.4 years (IQR: 1.1–6.2), and median number of visits was 6 (range: 1–17). The median time lag was 102 days (IQR: 86–183) between eCVL measurements and 181 days (IQR: 164–232) between plasma HIV-1 RNA measurements.

Detection of HIV-1 RNA in Plasma and Cervicovaginal Samples

Among the 174 women with available plasma samples, 90 (52%) had at least one detectable PVL on ART (Table 2). Twenty (11%) women experienced virologic treatment failure, and 27 (14%) changed regimen due to side effects or desire for pregnancy. PVL was detectable during 16% (171/1050; 95% CI: 14% to 19%) of visits, and the mean quantity of PVL during visits with detectable virus was 3.66 log₁₀ copies per milliliter (SD: ±1.05).

About two-thirds of women (68%, 128/188) had detectable cervicovaginal HIV-1 RNA at least once, and 4% shed at

all visits. Twenty-two percent (336/1498; 95% CI: 20% to 25%) of visits had detectable cervicovaginal HIV-1 RNA, with a mean quantity of 3.08 log₁₀ copies per milliliter (SD ±0.52) in detectable samples. Almost half of women (45%, 77/170) shed while having undetectable PVL, and 16% (128/798; 95% CI: 13% to 18%) of visits had detectable cervicovaginal HIV-1 RNA with undetectable PVL, with a mean quantity of 2.96 log₁₀ copies per milliliter (SD: ±0.36) in detectable samples. There was good correlation between plasma and cervicovaginal HIV-1 RNA during visits in which both were detectable, as indicated by Spearman's rank correlation coefficient of 0.47 ($P < 0.001$).

Association of Cervicovaginal HIV-1 RNA Detection With Behavioral and Biological Variables

There was little evidence that behavioral characteristics were associated with detectable cervicovaginal HIV-1 RNA (Table 3). There was weak evidence that high reported adherence (≥95%) was associated with lower odds of shedding (OR = 0.56, 95% CI: 0.30 to 1.05), and a trend toward increased odds of shedding for women using the injectable contraceptive depot medroxyprogesterone acetate (OR = 2.10, 95% CI: 0.91 to 4.85, compared with no hormonal contraception). These associations did not persist after adjustment for PVL.

In the univariable analysis of biological variables, several clinical factors were associated with cervicovaginal shedding (Table 4). The strongest associations were with concurrent *N. gonorrhoeae* infection (OR = 6.95, 95% CI: 1.58 to 30.62), detectable PVL (OR = 3.90, 95% CI: 2.48 to 6.13), presence of genital ulcers or vesicles (OR = 2.18, 95% CI: 1.26 to 3.78), and abnormal vaginal discharge on genital examination (OR = 1.84, 95% CI: 1.27 to 2.68). There was no evidence of an association of detectable cervicovaginal HIV-1 with detection of semen or cervicovaginal HSV-2 DNA. In terms of ART, current nevirapine use was associated with an increase in the odds of shedding (OR = 1.62, 95% CI: 1.11 to 2.38) compared with current use of efavirenz.

Adjustment for PVL weakened most associations. Abnormal vaginal discharge remained associated with HIV-1 RNA shedding, as did the impact of current nevirapine use. The use of a zidovudine-containing regimen was also predictive of shedding, compared with a stavudine-based regimen.

Multivariable Analysis of Detectable Cervicovaginal HIV-1 RNA

In the multivariable analysis of factors associated with detectable cervicovaginal HIV-1, which included adherence, PVL, and whether the sample was hemorrhagic, there was strong evidence that both current use of nevirapine [adjusted odds ratio (aOR) = 1.97, 95% CI: 1.23 to 3.15] and zidovudine (aOR = 2.11, 95% CI: 1.25 to 3.54) were predictive of shedding compared with efavirenz and stavudine, respectively. These results were conserved when we ran the model using ART regimen at the preceding visit. The association with abnormal vaginal discharge also persisted (aOR = 1.64, 95%

TABLE 2. Summary of Plasma and Cervicovaginal HIV-1 RNA Detection

Variable	Post-ART Initiation*
Median number of attended visits (range)	6 (1–17)
Analysis of plasma HIV-1 RNA	
Women with detectable plasma HIV-1 RNA, no. (%) (n = 174)	
Never	84 (48)
At any time	90 (52)
At all visits	15 (9)
Virologic treatment failure†	20 (11)
Proportion of visits with detectable plasma HIV-1 RNA (%)	171/1050 (16)
Mean quantity of plasma HIV-1 RNA in visits with detectable HIV-1 plasma RNA, log ₁₀ copies/mL (±SD)	3.66 (±1.05)
Analysis of cervicovaginal HIV-1 RNA	
Women with detectable cervicovaginal HIV-1 RNA, no. (%) (n = 188)	
Never	60 (32)
At any time	128 (68)
At all visits	8 (4)
While undetectable plasma HIV-1 RNA	77/170 (45)
Proportion of visits with detectable cervicovaginal HIV-1 RNA (%)	336/1498 (22)
Mean quantity of cervicovaginal HIV-1 RNA among visits with detectable cervicovaginal HIV-1 RNA—log ₁₀ copies/mL (±SD)	3.08 (±0.52)
Proportion of visits with detectable cervicovaginal HIV-1 RNA in visits with undetectable plasma HIV-1 RNA (95% CI)	128/798 (16)
Mean quantity of detectable cervicovaginal HIV-1 RNA in visits with undetectable plasma HIV-1 RNA—log ₁₀ copies/mL (±SD)	2.96 (±0.36)

*Denominators vary due to sample availability: Plasma viral loads were available for 174 women, and eCVLs for 188 women.

†Defined as plasma viral load >300 copies per milliliter after 6 months, or >300 copies per milliliter on 2 consecutive measurements after prior viral suppression.

TABLE 3. Demographic and Behavioral Factors and the Presence and Quantity of Cervicovaginal HIV-1 RNA During Visits on Antiretroviral Therapy

Characteristic	Cervicovaginal HIV-1 RNA Detected n/no. Visits (%)	Unadjusted Odds Ratio* for Detection of HIV-1 RNA (95% CI)		PVL-Adjusted Odds Ratio*† (95% CI)		Regression Coefficient ‡HIV-1 RNA Quantity (95% CI)		PVL-Adjusted Regression Coefficient‡‡ (95% CI)		
				P	P	P	P			
Age group§										
15–24 y	46/165 (28)	1.0		1.0		0		0		
25–34 y	153/705 (22)	0.73 (0.37 to 1.43)	0.51	0.94 (0.42 to 2.09)	0.84	–0.25 (–0.44 to –0.06)	0.009	–0.09 (–0.30 to 0.12)	0.61	
≥35 y	136/623 (22)	0.66 (0.33 to 1.33)		0.81 (0.36 to 1.82)		–0.31 (–0.50 to –0.11)		–0.11 (–0.32 to 0.11)		
Level of education§										
None	160/648 (25)	1.0		1.0		0		0		
Primary/secondary	175/845 (21)	0.87 (0.57 to 1.34)	0.54	0.78 (0.49 to 1.26)	0.32	–0.09 (–0.22 to 0.03)	0.15	–0.12 (–0.24 to 0.01)	0.08	
Adherence ≥95%	297/1371 (22)	0.56 (0.30 to 1.05)	0.08	0.90 (0.37 to 2.18)	0.83	–0.02 (–0.20 to 0.24)	0.85	0.19 (–0.10 to 0.49)	0.20	
Hormonal contraception										
None	267/1295 (21)	1.0		1.0		0		0		
Oral contraceptives	36/111 (32)	1.39 (0.80 to 2.42)	0.14	1.57 (0.75 to 3.27)	0.46	0.12 (–0.07 to 0.30)	0.15	0.04 (–0.18 to 0.26)	0.66	
DMPA	16/42 (38)	2.10 (0.91 to 4.85)	0.12	1.32 (0.42 to 4.16)	0.16	0.23 (–0.04 to 0.50)	0.80	–0.15 (–0.51 to 0.21)	0.62	
Regular vaginal douching	219/938 (23)	1.37 (0.92 to 2.05)		1.33 (0.82 to 2.18)		0.02 (–0.12 to 0.16)		–0.04 (–0.21 to 0.13)		
Number of sex acts in past week										
None	114/547 (21)	1.0		1.0		0		0		
1–4	114/511 (22)	1.09 (0.76 to 1.57)	0.40	1.61 (0.97 to 2.68)	0.19	0.07 (–0.07 to 0.20)	0.38	0.02 (–0.16 to 0.20)	0.35	
≥5	27/161 (17)	0.75 (0.43 to 1.33)		1.25 (0.60 to 2.62)		0.15 (–0.08 to 0.37)		0.19 (–0.08 to 0.46)		
Current condom use										
Never or rarely	128/566 (23)	1.0		1.0		0		0		
Frequently/Always	184/857 (21)	0.92 (0.68 to 1.26)	0.62	1.05 (0.70 to 1.57)	0.83	–0.02 (–0.14 to 0.09)	0.70	0.01 (–0.13 to 0.15)	0.87	

*Calculated using random-effects logistic regression, P-values determined by likelihood ratio tests.
 †Adjusted for plasma viral load as a categorical value (undetectable, 2.48–3.9, 4.0–4.9, ≥5.0 log₁₀ copies/mL).
 ‡Log₁₀ copies per milliliter; calculated using random-effects linear regression, The P values determined by Wald tests.
 §Determined at cohort enrollment; all other variables are concurrent to the cervicovaginal sample collection visit.
 DMPA, injectable depot medroxyprogesterone acetate.

CI: 1.01 to 2.66). The inclusion of contraception or pregnancy in the model did not impact the results and therefore they were excluded from the final model. There were not enough visits using PIs, tenofovir, emtricitabine, or abacavir to assess their impact on cervicovaginal HIV-1 shedding.

Factors Associated With the Quantity of Cervicovaginal HIV-1 RNA

Among those with detectable cervicovaginal HIV-1 RNA, increasing age was associated with a reduction in the quantity of cervicovaginal HIV-1 RNA (Table 3). There were no behavioral correlates of quantity of cervicovaginal HIV-1 RNA. The presence of cervicovaginal HSV-2 DNA was associated with a pronounced increase in cervicovaginal HIV-1 RNA quantity (regression coefficient 0.50 log₁₀ copies/mL, 95% CI: 0.26 to 0.75), as were abnormal discharge, an abnormal cervical exam, BV, and the use of nevirapine (Table 4). In the multiple linear regression model, the presence of BV (adjusted regression coefficient 0.15, 95% CI: –0.01 to 0.31), the presence of HSV-2 DNA (adjusted regression coefficient 0.29, 95% CI: 0.0 to 0.59), and the use of nevirapine (adjusted

regression coefficient 0.18, 95% CI: 0.03 to 0.33) were independently associated with an increase in cervicovaginal HIV-1 RNA quantity (Table 5). Including adherence in the model did not impact the results but reduced the power of the analysis due to missing data.

DISCUSSION

This is one of the most comprehensive longitudinal studies of cervicovaginal shedding of HIV-1 among women on ART to date. The study, among 188 African sex workers on ART followed for up to 8 years, found that cervicovaginal shedding occurred on 16% of visits in which the HIV-1 PVL was undetectable (95% CI: 13% to 18%), compared with 9% of visits found in a large study of women taking ART in the United States (95% CI: 6% to 14%).¹⁹ The mean quantity shed, 2.96 log₁₀ copies per milliliter, was in the range of quantities associated with female-to-male sexual transmission in PiP, where they observed 1.4 transmissions per 100 person-years (95% CI: 0.2 to 4.9) for women with endocervical HIV-1 concentrations less than 3 log₁₀ copies per swab.⁵ These results imply that, in this high-risk population, women on ART who

TABLE 4. Biological Factors and the Presence and Quantity of Cervicovaginal HIV-1 RNA During Visits on Antiretroviral Therapy

Characteristic	Cervicovaginal HIV-1 RNA Detected n/no. of Visits (%)	Unadjusted Odds Ratio* for Detection of HIV-1 RNA (95% CI)			PVL-Adjusted Odds Ratio*† (95% CI)			Regression Coefficient‡ HIV-1 RNA Quantity (95% CI)			PVL-Adjusted Regression coefficient†‡ (95% CI)		
				P		P		P		P			
Genital ulcers/ vesicles	31/84 (37)	2.18 (1.26 to 3.78)	0.01	1.28 (0.55 to 3.01)	0.57	0.16 (−0.03 to 0.35)	0.09	−0.10 (−0.38 to 0.17)	0.46				
Abnormal vaginal discharge	66/221 (30)	1.84 (1.27 to 2.68)	0.002	1.84 (1.16 to 2.90)	0.01	0.16 (0.02 to 0.30)	0.02	0.19 (0.04 to 0.34)	0.02				
Abnormal cervical exam	65/236 (28)	1.49 (0.99 to 2.25)	0.07	1.46 (0.87 to 2.45)	0.16	0.22 (0.07 to 0.36)	0.003	0.16 (−0.01 to 0.33)	0.06				
<i>N. gonorrhoeae</i>	7/11 (64)	6.95 (1.58 to 30.62)	0.008	1.88 (0.18 to 19.80)	0.60	−0.17 (−0.54 to 0.20)	0.37	−0.06 (−0.69 to 0.56)	0.84				
<i>T. vaginalis</i>	7/18 (39)	1.99 (0.65 to 6.04)	0.23	1.01 (0.28 to 3.73)	0.98	0.19 (−0.19 to 0.56)	0.34	0.34 (−0.08 to 0.75)	0.11				
<i>C. albicans</i>	16/80 (20)	0.95 (0.51 to 1.77)	0.87	1.20 (0.57 to 2.54)	0.63	−0.09 (−0.34 to 0.16)	0.47	−0.05 (−0.32 to 0.23)	0.75				
Bacterial vaginosis	100/485 (21)	0.79 (0.58 to 1.09)	0.17	0.76 (0.50 to 1.15)	0.19	0.15 (0.04 to 0.27)	0.01	0.15 (0.01 to 0.30)	0.04				
HSV-2 DNA positive	16/61 (26)	1.04 (0.52 to 2.07)	0.91	0.77 (0.32 to 1.88)	0.55	0.50 (0.26 to 0.75)	<0.001	0.34 (0.05 to 0.63)	0.02				
Y-PCR positive	21/100 (21)	0.86 (0.47 to 1.56)	0.59	1.09 (0.52 to 2.31)	0.82	−0.07 (−0.30 to 0.16)	0.56	−0.04 (−0.30 to 0.21)	0.73				
Hemorrhagic sample	23/71 (32)	1.81 (0.99 to 3.30)	0.05	1.94 (0.90 to 4.17)	0.10	0.09 (−0.13 to 0.30)	0.42	0.09 (−0.16 to 0.35)	0.48				
Current pregnancy	17/57 (6)	1.05 (0.95 to 1.16)	0.32	1.07 (0.93 to 1.23)	0.39	−0.01 (−0.04 to 0.03)	0.78	0.04 (−0.01 to 0.09)	0.11				
Plasma HIV-1 RNA detectable	69/158 (44)	3.90 (2.48 to 6.13)	<0.001	NA		0.28 (0.13 to 0.42)	<0.001	NA					
CD4+ count ≤100 cells/μl	12/28 (43)	2.10 (0.84 to 5.26)	0.12	1.50 (0.45 to 5.05)	0.54	0.11 (−0.19 to 0.41)	0.47	−0.10 (−0.44 to 0.25)	0.58				
Current ART regimen													
Efavirenz	141/770 (18)	1.0		1.0		0		0					
Nevirapine	162/653 (25)	1.62 (1.11 to 2.38)		1.61 (1.03 to 2.52)		0.14 (0.02 to 0.26)		0.16 (0.03 to 0.30)					
Protease Inhibitor	30/70 (43)	1.69 (0.79 to 3.64)	0.03	1.57 (0.59 to 4.13)	0.06	0.32 (0.11 to 0.53)	0.004	0.17 (−0.08 to 0.42)	0.04				
Current NRTI regimen													
Stavudine/lamivudine	108/527 (20)	1.0		1.0		0		0					
Zidovudine/lamivudine	206/918 (22)	1.18 (0.78 to 1.79)		1.65 (1.01 to 2.71)		−0.12 (−0.25 to 0.01)		−0.02 (−0.17 to 0.12)					
Other	19/48 (40)	1.09 (0.44 to 2.65)	0.89	1.17 (0.35 to 3.88)	0.11	0 (−0.26 to 0.26)	0.18	0.08 (−0.27 to 0.42)	0.83				
Time on ART													
0–6 months	38/159 (24)	1.0		1.0		0		0					
6–12 months	31/164 (19)	0.62 (0.34 to 1.13)		0.62 (0.29 to 1.34)		−0.03 (−0.27 to 0.21)		0.13 (−0.15 to 0.41)					
12–24 months	68/265 (26)	0.99 (0.58 to 1.68)		1.01 (0.50 to 2.03)		−0.08 (−0.28 to 0.12)		0.05 (−0.20 to 0.29)					
>24 months	196/905 (22)	0.86 (0.54 to 1.37)	0.29	0.77 (0.41 to 1.44)	0.45	−0.11 (−0.29 to 0.07)	0.62	0.09 (−0.13 to 0.32)	0.75				

*Calculated using random-effects logistic regression, P values determined by likelihood ratio tests.

†Adjusted for plasma viral load as a categorical value (undetectable, 2.48–3.9, 4.0–4.9, ≥5.0 log₁₀ copies/mL).

‡Log₁₀ copies per milliliter; Calculated using random-effects linear regression, P values determined by Wald tests.

NA, not applicable; NRTI, nucleoside reverse transcriptase inhibitor.

seem to be controlled might still be at risk of transmitting HIV to their sexual partners.

Nevirapine and zidovudine are the 2 antiretroviral compounds most widely used for the prevention of vertical transmission of HIV, and in our study were associated with higher rates of shedding than efavirenz-based or stavudine-based regimens. These results are unexpected as these compounds achieve higher concentrations in the female genital fluids than efavirenz or stavudine and must be interpreted with caution due to the potential for residual confounding in this observational study.^{34–36} However,

studies of drug regimen and seminal HIV-1 shedding have shown no association between drug penetration and local viral concentrations.^{37,38} Similarly, a cross-sectional study of women in the United States found that nonnucleoside reverse transcriptase inhibitor regimens were more likely than PI-based regimens to be associated with cervicovaginal shedding, although the latter also penetrate less into the genital tract.^{34,35,39} As the association between nevirapine use and increased shedding persisted after adjusting for PVL and adherence, it is possible that our measure of adherence was an overestimate due to social desirability bias. Thus, these

TABLE 5. Multivariable Analysis of Factors Associated With Quantity of Cervicovaginal HIV-1 RNA Although on Antiretroviral Therapy

Characteristic	Adjusted Regression Coefficient (95% CI)*	P
Level of education†		
None	0	0.08
Primary/Secondary	-0.13 (-0.27 to 0.02)	
Abnormal vaginal discharge	0.08 (-0.09 to 0.25)	0.33
Abnormal cervical exam	0.14 (-0.06 to 0.34)	0.16
Bacterial vaginosis	0.15 (-0.01 to 0.31)	0.06
HSV-2 DNA positive	0.29 (0 to 0.59)	0.05
Plasma viral load (log ₁₀ copies/mL)		
Undetectable	0	<0.001
2.48–3.9	0 (-0.18 to 0.18)	
4.0–4.9	0.38 (0.12 to 0.65)	
≥5.0	0.78 (0.50 to 1.05)	
Current ART regimen		
Efavirenz	0	0.05
Nevirapine	0.18 (0.03 to 0.33)	
Protease Inhibitor	0.15 (-0.12 to 0.42)	

*Log₁₀ copies per milliliter. Adjusted for all variables in the table. Calculated using random-effects linear regression, P values determined by Wald test.

†Determined at cohort enrollment; all other variables are concurrent to the cervicovaginal sample collection visit.

data might reflect that adherence is lower for nevirapine-based regimens, confirming the results seen in a cohort of FSWs taking nevirapine-based regimens in Kenya, in whom lower reported adherence predicted cervicovaginal shedding even in those with suppressed plasma HIV-1 RNA.¹⁷ Although there was no association between these regimens and virologic treatment failure in our study, multiple studies have identified the virologic inferiority of nevirapine-based regimens.^{40–42} Furthermore, the majority of patients in the trial of ART as prevention were on efavirenz-based regimens (72%).⁸ Studies examining the impact of different ART regimens on the development of resistance in the genital compartment could help elucidate potential causes for this association, as could studies providing more data on intracellular concentrations of antiretrovirals in the genital tract, which might better reflect antiviral potential.³⁶

We found that in visits including those without PVL suppression, factors associated with mucosal inflammation, such as infection with *N. gonorrhoeae*, or disruption, such as ulcerative diseases, were strongly associated with a risk of cervicovaginal shedding. In our final model, abnormal vaginal discharge remained an independent correlate of shedding. It confirms results from cohort studies of women in the United States and Kenya where genital HIV detection was associated with increased cervical white blood cell infiltrates and cervicitis.^{43,44} Although our results do not provide specific information on causes of compartmentalized replication of HIV-1, they underscore the role of potential drivers of onward transmission in a setting of expanded ART.

Furthermore, although neither HSV-2 shedding nor BV were associated with an increased risk of HIV-1 shedding, they were independently associated with an increase in quantity of cervicovaginal HIV-1 RNA. This is the first study to demonstrate this effect in women on ART, although the impact of HSV-2 on the genital quantity of HIV-1 has been shown in ART-naive women, and is thought to be secondary to the genital mucosa enabling increased local replication of HIV-1.^{45,46} HSV-2 is thought to have been a significant contributor to the HIV-1 epidemic in sub-Saharan Africa,^{47,48} although trials found no effect of HSV suppressive therapy on HIV-1 transmission or acquisition.^{49,50} BV has been correlated with an increased quantity of genital HIV-1 RNA in ART-naive women¹³ and was associated with a 3-fold increase in female-to-male transmission of HIV-1 in untreated women in Africa.⁵¹ These increases in quantity on ART are probably too small to influence sexual transmission risk⁴⁹ although it is possible that there might be subpopulations of women who are frequent shedders who might benefit from adjunct therapies in conjunction with ART.⁵²

In light of the increased risk of transmission of HIV-1 in women using depot medroxyprogesterone acetate seen in PiP,⁵³ it is reassuring that we did not identify any association of hormonal contraception with shedding in women with suppressed PVL, indicating that effective ART might diminish any risk. Furthermore, we did not find any increase in shedding over time, supporting the durability of protectiveness of ART.

Limitations of our study include a less sensitive assay than used in high-income settings for HIV-1 RNA detection for eCVL and PVL, which did not allow us to determine if low levels of plasma viremia were contributing to the observed shedding. However, the higher threshold for detection would be more likely to underestimate the frequency of shedding. Furthermore, eCVL samples contain both cell-free and cell-associated HIV-1 RNA and DNA. Particularly in the cases of mucosal inflammation and vaginal discharge, the possibility that the majority of detected HIV-1 is present in inflammatory cells that have migrated to the mucosa requires further study.⁴³ However, as HIV-1 can be cultured from cell-free and cell-associated specimens, their relative importance in sexual transmission remains unclear.⁵⁴

The lower frequency of PVL measurements also reduced the power of our study to examine mucosal correlates of shedding. It is possible that there are subtle factors that would require more frequent sampling in both compartments for their identification. It has been shown that there is greater within-woman variability in quantities of genital rather than plasma HIV-1 RNA, which informed the differential frequency of sampling of each compartment in our study.⁵⁵ Finally, although we have a better understanding of genital HIV-1 shedding and sexual transmission risk based on the data from the PiP trial, the women in that study were not on ART; thus we do not have a reliable threshold of infectivity on ART. Further information on infectivity might be drawn from additional trials of ART as prevention, particularly those including high-risk groups in their design. Conclusions from our study are thus not definitive but do advance understanding of the role of ART in prevention in different settings in Africa.

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APPENDIX 1. Composition of the Yérelon Study Group

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