Prevalence, Magnitude, and Correlates of HIV-1 Genital Shedding in Women on Antiretroviral Therapy

Caroline C. King,[1] Sascha R. Ellington,[1] Nicole L. Davis,[1] Robert W. Coombs,[2,3] Maria Pyra,[4,5] Ting Hong,[5] Nelly Mugo,[4,5] Rena C. Patel,[3] Jairam R. Lingappa,[3,5] Jared M. Baeten,[3,4,5] and Athena P. Kourtis[1]; on behalf of the Partners in Prevention HSV/HIV Transmission Study and Partners PrEP Study Teams

[1]Division of Reproductive Health, Centers for Disease Control and Prevention, Atlanta, Georgia; Departments of [2]Laboratory Medicine, [3]Medicine, [4]Epidemiology, and [5]Global Health, University of Washington, Seattle

(See the editorial commentary by Sivro and McKinnon on pages 1484–6.)

Background. Genital human immunodeficiency virus (HIV) RNA shedding can continue despite HIV being undetectable in blood, and can be associated with transmission.

Methods. We included African women on antiretroviral therapy (ART). Linear and generalized linear mixed models were used to compare the magnitude and prevalence of genital shedding, respectively, by time since ART initiation. Multivariable logistic regression with generalized estimating equations was used to assess predictors of genital shedding among women with undetectable plasma viral load (VL).

Results. Among 1114 women, 5.8% of visits with undetectable plasma VL and 23.6% of visits with detectable VL had genital shedding. The proportion of visits with genital shedding decreased with time since ART initiation but the magnitude of shedding remained unchanged when plasma VL was undetectable ($P = .032$). Prevalence of shedding did not vary by time since ART initiation when plasma VL was detectable ($P = .195$), though the magnitude of shedding significantly increased ($P = .04$). Predictors of genital shedding were HIV disease stage, antiretroviral regimen, and genital ulcers or cervical tenderness.

Discussion. In addition to ART, reducing immune activation through prevention and treatment of HIV-related conditions and genital tract infections may decrease the risk of HIV-1 shedding and potential transmission.

Keywords. antiretroviral; genital shedding; HIV; viral load.

Use of antiretroviral drugs is highly effective for both treatment of human immunodeficiency virus (HIV) infection and the prevention of sexual and mother-to-infant HIV transmission [1, 2]. However, while antiretroviral therapy (ART) initiation is associated with a rapid decline in plasma viral load (VL) and genital shedding of HIV RNA [3], some women on stable ART continue to have detectable levels of HIV RNA in cervicovaginal secretions, often intermittently [4]. Most HIV transmission occurs via exposure to genital secretions, and genital viral load (GVL) is a predictor of sexual and mother-to-child HIV transmission independent of plasma VL [5]. The reasons for ongoing HIV genital shedding in the context of effective ART and undetectable plasma VL are unknown but likely involve local genital factors that potentiate viral shedding.

Among ART-naive women, determinants of genital HIV shedding include plasma VL and factors associated with mucosal inflammation (eg, *Neisseria gonorrhoeae* infection) or disruption (eg,

genital ulcer disease) [6, 7]. However, studies among women on ART have reported inconsistent findings on the role of genital infections on genital HIV shedding [6, 8, 9]. A further complication of the role of ART on shedding is that ART regimen type has been associated with shedding, and certain ART compounds, such as nevirapine and zidovudine, achieve higher concentrations in the female genital tract than others, such as efavirenz and stavudine [6, 10–12]. Effective penetration of ART into the genital tract may counteract other factors associated with genital HIV shedding in ART-naive women. Furthermore, shedding among women on ART may be further affected by menses [13] and also by potential drug–drug interactions with progestin-based contraceptive use, which has been implicated in potentially increasing risk of HIV shedding and transmission among ART-naive populations [14, 15].

In the current study, we aimed to understand the prevalence, magnitude, and correlates of HIV genital shedding among 1114 women on ART, overall and among those with undetectable plasma VL, to better understand potential drivers of HIV transmission in a setting of expanded ART.

METHODS

Study Background and Population

The analysis used data from HIV-infected women enrolled in 3 prospective HIV transmission studies (Partners in Prevention HSV/HIV Transmission Study [PiP], Couples Observation Study,

and Partners PrEP Study) [14, 16, 17]. These studies enrolled 8640 HIV-serodiscordant heterosexual couples from 7 African countries (Botswana, Kenya, Rwanda, South Africa, Tanzania, Uganda, and Zambia) from 2004 to 2013 and followed them for 12–48 months. The data collection instruments and sample collection practices were coordinated to collect similar demographic, behavioral, clinical, and laboratory information and permit merging of the data across studies to increase statistical power. Demographic information was collected at enrollment, and data on sexual behavior, contraceptive use, and self-reported ART use were collected quarterly. CD4+ T-cell count and plasma HIV RNA level were measured every 6 months. Genital tract HIV shedding was measured every 6 months in 2 studies and annually in the Partners PrEP Study. HIV RNA was quantified in blood and endocervical swab samples using the COBAS AmpliPrep/ COBAS TaqMan real-time HIV RNA assay version 1.0 (Roche Diagnostics) or the Abbott m2000 Real-Time HIV assay (Abbott Diagnostics), with the lowest limit of detection at 40 copies/mL in blood and 240 copies/swab in endocervical swabs. At enrollment, none of the HIV-infected serodiscordant partners (63% female) were on ART, but their clinical and immunological status were monitored during follow-up, and they received referrals for ART if they met national guidelines to initiate ART. All couples were aware of their serodiscordant status and received individual and couples HIV risk-reduction counseling as well as prevention services, including free condoms and screening and treatment for sexually transmitted infections, according to World Health Organization (WHO) guidelines. The studies were approved by the University of Washington Human Subjects Review Committee and local ethics review boards associated with each study site. All women provided informed, written consent.

Analysis

The analysis was limited to women who initiated ART. Date of ART initiation was back-estimated from the first visit at which ART use was reported, using either the self-reported number of days of ART use or the midpoint from the prior visit interval. Follow-up was censored at the first report of no longer taking antiretrovirals. Plasma and genital HIV RNA concentrations were $\log_{10}$-transformed to approximate normality. Samples below the limit of quantification were assigned values at half that limit and were considered as having undetectable plasma VL or as having no shedding.

Among women who initiated ART, we assessed the prevalence and magnitude of HIV-1 RNA shedding (percent of visits with shedding and mean HIV RNA concentration, respectively) in endocervical samples from visits with detectable and undetectable plasma VL. We further stratified the shedding prevalence into 3 time intervals (<6, 6–12, and >12 months) since ART initiation and compared these estimates using generalized linear mixed models to account for potential repeated visits within a time period. Similarly, we used linear mixed models to estimate and compare the magnitude of shedding across time period since ART initiation. All models were adjusted for a combined variable of study and randomization to daily acyclovir in the PiP study. Among women with more than one endocervical swab after ART initiation, we evaluated the degree to which women never shed, always shed, or had visits of both shedding and no shedding, and compared the mean GVL at the first visit among women who continued to shed HIV to those who did not, using an independent t test. Among women with at least 2 GVL assessments who were not shedding at the first visit and had consistently undetectable plasma VL, we estimated the cumulative incidence of a new shedding visit by time since ART initiation.

We evaluated potential predictors of genital HIV shedding (shedding versus no shedding) among women with undetectable plasma VL, censoring follow-up at the visit prior to a detectable plasma VL. We used logistic regression with generalized estimating equations and an unstructured correlation matrix, and adjusted for a combined variable of study and randomization to daily acyclovir in one of these studies [17]. Potential predictors included demographic factors (age, education, having any monthly income, marital status), immunological indicators (CD4+ T-cell count, WHO stage), antiretroviral factors (regimen and duration), genital tract infections (*Trichomonas vaginalis, N. gonorrhoeae, Chlamydia trachomatis, Treponema pallidum*), genital conditions noted on examination (genital ulcers or cervical bleeding, mucous, discharge, or tenderness), behavioral risk factors (any unprotected sex acts in previous month, uncircumcised partner), and hormonal factors (contraceptive type, pregnancy). Data on bacterial vaginosis were not collected in the Couples Observation Study, and serologic evidence of herpes simplex type 2 (HSV-2) infection was not collected consistently in HIV-infected subjects in the Partners PrEP Study, therefore these potential predictors of HIV shedding could not be evaluated in the combined population. Separate multivariable models were built for each predictor and the outcome of shedding, and factors were considered for adjustment in these models based on whether they changed the predictor's effect estimate by >10% or improved precision. Due to the stability of predictor associations with shedding across different multivariable models and increased precision with multiple adjustment, we present simplified results for a single final model that adjusts for age and all factors suggestive of an association in both univariate and multivariable analysis.

RESULTS

Of the 1114 women who had a GVL measurement while on ART, most (96.1%) were married to their study partner (Table 1). At the most recent visit prior to first reported ART use, the median age was 32 years (interquartile range [IQR], 27–38), the median CD4+ count was 274 cells/μL (IQR, 211–371), and the median plasma HIV RNA concentration was 4.3 $\log_{10}$ copies/mL (IQR,

Table 1. Baseline Characteristics of 1114 HIV-Infected African Women

Characteristic	Median (IQR) or n (%)
Age (years)[a]	32 (27–38)
Married to study partner	1071 (96.1)
Education (years)	7 (4–9)
Parity	
0	99 (8.9)
1–2	462 (41.5)
3–4	344 (30.9)
5+	209 (18.7)
Any monthly income	574 (51.5)
CD4+ T-cell count, cells/μL[a]	274 (211–371)
Plasma HIV RNA level, $\log_{10}$ copies/mL[a]	4.3 (3.5–4.8)
WHO disease stage[a]	
1	413 (37.1)
2	556 (49.9)
3	136 (12.2)
4	9 (0.8)
Sexually transmitted pathogen[a]	
Trichomonas vaginalis	95 (8.7)
Neisseria gonorrhoeae	17 (1.6)
Chlamydia trachomatis	5 (0.5)
Treponema pallidum	22 (2.0)
Genital conditions noted on examination[a]:	
Genital ulcer	34 (3.1)
Cervical bleeding, mucous, or discharge	248 (22.5)
Cervical tenderness	28 (2.5)
Any unprotected sex in the preceding 3 months[a]	183 (16.4)
Uncircumcised partner	522 (46.8)
Pregnancy[a]	73 (6.6)
Hormonal contraceptive method[a]:	
Injection	203 (18.2)
Implant	61 (5.5)
Oral	60 (5.4)
None	790 (70.9)
Antiretroviral therapy[b]:	
Efavirenz based	182 (16.3)
Nevirapine based	798 (71.6)
PI based	17 (1.5)
Other or unknown	117 (10.5)
Duration, months[c]	5.0 (2.4–9.7)

Missing data: plasma HIV RNA level = 9; *Trichomonas vaginalis* = 27; *Neisseria gonorrhoeae* = 35; *Chlamydia trachomatis* = 35; *Treponema pallidum* = 9; ulcer = 12; cervical bleeding, mucous, or discharge = 13; cervical tenderness = 13.

Abbreviations: IQR, interquartile range; WHO, World Health Organization; ART, antiretroviral therapy; PI, protease inhibitor.

[a]Time-varying characteristics are from last assessment prior to ART initiation.

[b]Regimen at the first measurement of genital viral load while on ART. Other/unknown ART regimen includes 116 unknown and 1 nucleoside reverse transcriptase inhibitors-based regimen.

[c]Median duration from estimated ART initiation to the first genital viral load assessment.

3.5–4.8); 145 (13.0%) were at WHO clinical stage 3 or 4. The ART regimen at the first GVL assessment was nevirapine-based for 798 (71.6%), efavirenz-based for 182 (16.3%), protease inhibitor (PI)-based for 17 (1.5%), and other/unknown for 117 (10.5%) women (Table 1).

After ART initiation, GVL levels were available at 1 time point for 595 women (53.4%) and 2, 3, and 4 time points for 330

(29.6%), 165 (14.8%), and 24 (2.2%) women, respectively. The median time from ART initiation to the first GVL assessment was 5.0 (IQR, 2.4–9.7) months, at which 90.0% (n = 1002) of women had undetectable GVL.

Despite the significant correlation between plasma and genital VL (Pearson correlation coefficient 0.27, *P* < .001), genital HIV RNA was detected at 5.8% (83 visits from 76 women) of 1433 visits with undetectable plasma VL, and the mean GVL at these visits was 3.21 (standard deviation [SD] = 0.48) $\log_{10}$ copies/ swab. In comparison, when plasma VL was detectable (377 visits), genital shedding was detected at 23.6% (89 visits from 77 women) of visits, with a mean GVL of 3.59 (SD = 0.69) $\log_{10}$ copies/swab (*P* = .001 for comparison of mean GVL). Among visits with undetectable plasma VL, the proportion with genital shedding decreased by time interval since ART initiation; shedding occurred at 8.3% of visits <6 months from ART initiation, 5.1% at 6–12 months, and 4.6% at >12 months (*P* = .032; Table 2). However, the mean GVL remained near 3.1 copies/ swab across the 3 time periods. In contrast, among visits with detectable plasma VL, the proportion shedding did not vary by time since ART initiation (*P* = .195), but the magnitude of GVL significantly increased with time since estimated ART initiation (*P* = .040; Table 2).

Among the 412 women with multiple assessments of GVL while having consistently undetectable plasma VL, 89.6% (n = 369) never shed, 0.7% (n = 3) always shed, and 9.7% (n = 40) had visits with and without shedding. Of the 40 women with both detectable and undetectable GVL across 2 to 4 visits, 19 (47.5%) went from shedding to clearing, 19 (47.5%) went from clear to shedding, and 2 (5%) had intervals of both clearing and new shedding of genital HIV. The 19 women who went from shedding to clearing the virus had a lower mean GVL at their first visit after ART initiation (3.04 [SD = 0.44] copies/swab) compared to the 3 women who always shed (3.96 [SD = 0.33] copies/swab) (*P* = .003). Among the 389 women with consistently undetectable plasma VL and undetectable GVL at their first assessment, the estimated cumulative incidence of a new shedding visit by time since ART initiation was 0.6% (95% confidence interval [CI], 0.2–1.9) at 12 months, 2.3% (95% CI, 1.2–4.4) from 12 to 18 months, 7.5% (95% CI, 4.4–12.5) from 18 to 24, and 16.8% (95% CI, 6.3–40.8) from 24 to 36 months.

Predictors of genital HIV shedding among 900 women with undetectable plasma VL were consistent in univariate and multivariable analyses and included WHO classification of HIV disease stage, type of antiretroviral regimen, and presence of genital ulcers or cervical tenderness (Table 3). Women in a higher WHO stage had increased odds of genital HIV shedding compared to stage 1: stage 2 adjusted odds ratio (aOR) = 2.67 (95% CI, 1.30–5.50); stage 3 aOR = 2.16 (95% CI, 0.91–5.16); stage 4 aOR = 8.74 (95% CI, 2.63–29.0). Women on efavirenz-based ART were significantly less likely to have genital HIV shedding compared to women on PI-based regimens (aOR

Table 2. Cervical HIV RNA Shedding by Time Since ART Initiation

	Time Since ART Initiation			
	<6 months	6–12 months	>12 months	P value[a]
Detectable plasma VL (377 visits, 309 women)				
Shedding, % (n/N visits)	20.6 (40/194)	27.4 (17/62)	26.5 (32/121)	.195
Log$_{10}$ genital VL among shedders, mean (95% CI)[a]	3.42 (3.13–3.70)	3.75 (3.36–4.13)	3.82 (3.44–4.19)	.040
Undetectable plasma VL (1433 visits, 900 women)				
Shedding, % (n/N visits)	8.3 (33/398)	5.1 (23/449)	4.6 (27/586)	.032
Log$_{10}$ genital VL among shedders, mean (95% CI)[a]	3.17 (2.97–3.37)	3.16 (2.92–3.34)	3.10 (2.86–3.33)	.536

Missing data: for 36 visits with a genital VL assessment, there was no plasma VL measurement within 30 days.

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; n, number of visits with shedding; N, total number of visits; VL, viral load.

[a] To account for repeated measures within intervals, P values were derived from generalized linear mixed models with a binary distribution for the outcome of shedding versus no shedding and P values and estimated mean log genital VLs were derived from linear mixed models. Models were adjusted for a combined variable of study (Partners in Prevention [PiP], Couples Observation Study, and Partners PrEP Study) and randomization to acyclovir in PiP.

= 0.20, 95% CI, 0.05–0.78) (not shown) or unknown/other regimens (aOR = 0.33, 95% CI, 0.13–0.83) (not shown), but not when compared to women on nevirapine-based regimens (aOR = 0.62, 95% CI, 0.31–1.23). Presence of genital ulcers (aOR = 3.26, 95% CI, 1.04–10.2) and cervical tenderness (aOR = 3.54, 95% CI, 1.03–12.2) on examination were associated with increased odds of genital HIV shedding. Pregnancy and reported hormonal contraceptive use were not associated with genital HIV shedding among the women (Table 3).

DISCUSSION

In this large, prospective cohort of HIV-infected women on ART, genital HIV shedding was very low and occurred at only 6% of visits when plasma VL was undetectable. This percentage was lower than other studies of women on ART with VLs <500 copies/mL or undetectable: 9% among a longitudinal study of 59 US women [4], 15% among a cross-sectional study of 290 US women [18], and 16% among a longitudinal study of 188 women from Burkina Faso [6]. However, studies of smaller sample size, less consistent ART use, and different assay sensitivities have estimated a wide range (0–40%) of shedding among women with suppressed plasma VL [9, 19, 20]. Among women with genital HIV shedding in our study, the median GVL among both those with undetectable (3.16 copies/swab) and detectable (3.50 copies/swab) plasma VL was in the range of quantities associated with an estimated risk of female-to-male sexual transmission at 2.0 transmissions per 100 person-years (95% CI, 1.2–3.1) [21]. While we did not observe any cases of sexual HIV transmission among women included in this analysis with undetectable plasma VL who were on ART, these results suggest that women on ART may still be at some risk of transmitting the virus despite effective ART and relatively low prevalence and magnitude of shedding.

Among women with undetectable plasma VL, we found predictors of genital HIV shedding that may provide further insight into the woman's systemic or genital immunologic and virologic status. Later WHO HIV disease stage indicates worsening systemic immunologic status and may better indicate immune activation through the presence of HIV-related conditions or coinfections than CD4$^+$ count, comprised of activated and resting CD4$^+$ T cells. In our study and some others, CD4$^+$ count was no longer associated with genital HIV shedding after accounting for plasma VL [9, 19, 20]. Furthermore, previous studies have indicated that systemic immune activation levels can reflect local immunologic activation that may promote genital HIV replication [22, 23]. Other factors that may be indicative of local immune activation were associated with shedding, such as genital ulcerations. Genital ulcerations may increase vascular permeability, allowing transmigration from the bloodstream to the cervicovaginal compartment, or activate HIV-replication by infiltration of activated lymphocytes and monocytes and production of inflammatory mediators locally in the genital tract [24–26]. Cervical tenderness was also significantly associated with shedding and may be indicative of cervicitis or an active cervical infection. A longitudinal study of HIV RNA on cervical swabs collected across precervicitis, cervicitis, and postcervicitis visits among women on ART showed an increase in genital HIV RNA with cervicitis and a decrease post-treatment [27]. In our study, the prevalence of cervical infections, such as chlamydia (0.5%) and gonorrhea (1.6%), were low, and were not significantly associated with shedding among women with undetectable plasma VL, possibly due to limited power to detect a difference given the small sample of women with undetectable plasma VL, genital HIV shedding, and cervical infections. However, these findings are consistent with other studies that found no significant association with genital tract infections among women on suppressive ART [6, 8].

Genital HIV shedding with undetectable plasma VLs may also be attributable to HIV compartmentalization in the genital tract and isolated viral replication due to incomplete penetration of antiretroviral drugs [28]. Among women with undetectable plasma VL, we found that efavirenz-based ART regimens were significantly associated with lower odds of genital HIV

Table 3. Associations With Genital HIV RNA Detection Among Women With Undetectable Plasma Viral Load

Characteristic	Unadjusted[a] OR (95% CI)	Adjusted[b] OR (95% CI)
Age (years)	1.02 (0.99–1.05)	1.03 (1.00–1.06)
Married to study partner	1.10 (0.27–4.49)	…
Education (years)	0.98 (0.92–1.04)	…
CD4+ T-cell count, 100 cells/µL[a]	0.95 (0.85–1.07)	…
WHO stage		
1	Ref.	
2	2.50 (1.25–5.00)	2.67 (1.30–5.50)
3	1.96 (0.82–4.72)	2.16 (0.91–5.16)
4	7.74 (2.08–28.9)	8.74 (2.63–29.0)
Sexually transmitted pathogen[a]:		
Trichomonas vaginalis	1.27 (0.53–3.07)	…
Neisseria gonorrhoeae	1.82 (0.45–7.34)	…
Treponema pallidum	…	…
Genital conditions noted on examination:		
Genital ulcer	4.01 (1.18–13.6)	3.26 (1.04–10.2)
Cervical bleeding, mucous or discharge	1.39 (0.73–2.66)	…
Cervical tenderness	3.62 (1.07–12.2)	3.54 (1.03–12.2)
Any unprotected sex[a]	0.69 (0.34–1.41)	…
Uncircumcised partner	1.02 (0.64–1.63)	…
Pregnancy	0.97 (0.36–2.64)	…
Hormonal contraceptive method:		
Injection	0.98 (0.57–1.69)	…
Implant	0.76 (0.27–2.19)	…
Oral	1.23 (0.47–3.25)	…
None	Ref.	
Antiretroviral therapy[b]:		
Nevirapine based	Ref.	Ref.
Efavirenz based	0.71 (0.36–1.40)	0.62 (0.31–1.23)
PI based	1.77 (0.91–3.48)	1.87 (0.91–3.87)
Other or unknown	2.68 (0.58–12.4)	3.16 (0.93–10.7)
Duration, months	0.99 (0.96–1.02)	…

Abbreviations: OR, odds ratio; CI, confidence interval; WHO, World Health Organization; PI, protease inhibitor; Ref, referent.

[a]Models were adjusted for a combined variable of study (Partners in Prevention [PiP], Couples Observation Study, and Partners PrEP Study) and randomization to acyclovir in PiP.

[b]Models were adjusted for a combined study and acyclovir variable and all factors shown.

shedding compared to PI-based regimens and other/unknown regimens, but they were not appreciably different from nevirapine-based regimens. Despite studies reporting that both PIs and efavirenz achieve lower concentrations in the genital tract compared to nevirapine and nucleoside reverse transcriptase inhibitors [11, 12], another study reported the unexpected finding that nevirapine and zidovudine were associated with higher rates of genital HIV shedding compared to efavirenz-based or stavudine-based regimens, even after adjustment for plasma VL and other factors [6]. In another study, despite low levels of PIs and nonnucleoside reverse transcriptase inhibitors in the cervicovaginal fluid relative to in plasma, sustained suppression of HIV RNA levels was seen in the genital tract [12]. Factors beyond the level of antiretroviral penetration into the genital tract, such as efficacy of antiretrovirals at the systemic or local level, may contribute to our findings.

This analysis of 3 large, prospective, multinational cohorts was limited by self-report of ART and lack of information on ART adherence, limitations that could potentially be overcome in future studies by measurement of antiretroviral medications in plasma. Furthermore, for the analysis of predictors of genital HIV shedding among women with undetectable plasma VL, we were unable to evaluate and control for potential factors that were not consistently collected across the 3 combined studies, such as HSV-2 status and bacterial vaginosis. Other studies among ART-naive populations have reported inconsistent findings with regards to a role of bacterial vaginosis on shedding. While HSV-2 and suppressive treatment for HSV-2 have been shown to affect HIV shedding, the main mechanism for increased shedding and transmission is likely through genital ulceration, which, even though it underestimates the number with active HSV-2 infection, was evaluated and was a strong predictor in this study,

As therapeutic ART is increasingly relied upon as a prevention tool, clinicians may not be able to rely solely on confirmed plasma VL suppression to eliminate concern of HIV transmission. Our study suggests that in addition to suppressive ART, reducing systemic or local immune activation through prevention and treatment of HIV-related conditions or coinfections and genital tract infections that present as genital ulcers or cervical tenderness may further reduce any potential risk of HIV shedding and transmission of HIV-1 to partners and offspring.

Notes

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