BRIEF REPORT

Antiretroviral Adherence and Development of Drug Resistance Are the Strongest Predictors of Genital HIV-1 Shedding among Women Initiating Treatment

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Persistent genital human immunodeficiency virus type 1 (HIV-1) shedding among women receiving antiretroviral therapy (ART) may present a transmission risk. We investigated the associations between genital HIV-1 suppression after ART initiation and adherence, resistance, pretreatment CD4 cell count, and hormonal contraceptive use. First-line ART was initiated in 102 women. Plasma and genital HIV-1 RNA levels were measured at months 0, 3, and 6. Adherence was a strong and consistent predictor of genital HIV-1 suppression ($P < .001$), whereas genotypic resistance was associated with higher vaginal HIV-1 RNA level at month 6 ($P = .04$). These results emphasize the importance of adherence to optimize the potential benefits of ART for reducing HIV-1 transmission risk.

Antiretroviral therapy (ART) has improved the health of millions of women living with human immunodeficiency virus type 1 (HIV-1) infection [1]. In addition to providing individual benefits, ART greatly reduces plasma and genital viral load [2, 3] and has been associated with decreased sexual HIV-1 transmission [4, 5]. Nonetheless, continued genital shedding by women receiving ART [6], including shedding of drug-resistant virus [7], has been documented. Because of increasing interest in ART for reducing sexual transmission of HIV-1 [8], data regarding the prevalence and correlates of genital HIV-1 shedding among treated individuals are needed to inform the development of novel prevention interventions.

ART regimens containing nonnucleoside reverse transcriptase inhibitors (NNRTIs) are the most commonly used worldwide. A comprehensive, prospective evaluation of the effect of such regimens on genital HIV-1 shedding is timely. This study aimed to determine whether genital HIV-1 shedding among treated women was influenced by cofactors associated with shedding in the absence of ART [9] or was primarily influenced by cofactors known to predict plasma viral load suppression. We hypothesized that better adherence, absence of genotypic resistance, higher pretreatment CD4 cell count, and absence of hormonal contraceptive use would be associated with more effective suppression of genital HIV-1 among women initiating ART.

Methods. HIV-1-seropositive, nonpregnant women were invited to participate if they were eligible for ART according to Kenyan National Guidelines (CD4 cell count of ≤200 cells/mL or AIDS-defining illness) and willing to undergo monthly follow-up. All participants gave written informed consent. Ethical review committees of the Kenya Medical Research Institute and University of Washington approved the study.

The standard ART regimen was stavudine, lamivudine, and nevirapine, in accordance with World Health Organization (WHO) and Kenyan National Guidelines at the time [10]. During month 1, the receipt of 1 dose was directly observed each weekday. Pill box organizers were used to promote adherence, which was monitored at each visit by pill count ([no. of pills taken divided by total no. expected] ×100%). If the pill box was unavailable, adherence was calculated on the basis of patient recall of pills taken.

Women were screened for genital infections prior to ART initiation. At the baseline and monthly thereafter, women were interviewed using standardized questionnaires about sexual behavior, contraceptive practices, and genitourinary symptoms. A pelvic speculum examination was performed, which included collection of specimens for diagnosis of genital infections and viral quantitation, according to methods published elsewhere [3, 11]. If women were menstruating, the examination was re-

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scheduled. Blood was collected for HIV-1 quantitation at the baseline and quarterly thereafter.

After Gram staining of cervical secretion samples, polymorphonuclear leukocytes were counted in 3 nonadjacent oil immersion fields on microscopic analysis. Yeast and *Trichomonas vaginalis* were detected by microscopic analysis of wet preparations. The presence of bacterial vaginosis (BV) was evaluated by Nugent scoring of vaginal Gram stains. Sperm on the cervical Gram stain or vaginal wet preparation were noted. Culture for *Neisseria gonorrhoeae* was performed on modified Thayer-Martin medium. Nucleic acid amplification assays (Aptima Combo 2; Gen-Probe) were used to detect *N. gonorrhoeae* or *Chlamydia trachomatis*. Rapid β–human chorionic gonadotropin tests were used to detect pregnancy (Plasmatec Laboratory Products).

CD4 cell counts were determined using an automated method (FACSCount; Becton Dickinson). Plasma samples were frozen at −70°C until shipment to the University of Washington (Seattle, WA) for HIV-1 RNA quantitation using the Gen-Probe HIV-1 viral load assay [12]. The lower limit of quantitation was 100 copies per swab in genital secretion samples and 100 copies/mL in plasma samples. Genotypic resistance was evaluated in all plasma samples collected at month 6 in which an HIV-1 RNA level of >1000 copies/mL was detected. A nested real-time polymerase chain reaction (PCR) method designed for HIV-1 subtypes common in Kenya was used to amplify an ~800-bp fragment of reverse transcriptase [13]. Product sequences of 2 independent real-time PCR analyses were evaluated; additional real-time PCR analyses were performed as needed to verify mutations detected in only 1 of 2 initial PCR analyses. The Stanford University HIV Drug Resistance Database (http://hivdb.stanford.edu) was used to identify drug resistance mutations. If viral complementary DNA could not be amplified, we assumed that no drug-resistant variants were present.

The analysis used the intent-to-treat principle, which included women who changed or discontinued ART. The primary analysis made use of multivariate linear regression to determine the independent effects of each cofactor on the change in genital HIV-1 RNA level from month 0 to month 3 and from month 0 to month 6. Separate analyses were conducted for cervical and vaginal HIV-1 RNA shedding. Cofactors of interest included adherence (averaged over the period), genotypic antiretroviral resistance (measured at month 6), pretreatment CD4 cell count (<100 vs ⩾100 cells/μL), and hormonal contraceptive use (any exposure within 70 d). Genital ulcers and infections including syphilis, cervicitis, BV, trichomoniasis, and candidiasis were evaluated as potential confounding factors. Sensitivity analyses investigated the effects of recall adherence, visible blood on swabs, semen detection, and menstrual status on results.

For samples with HIV-1 RNA levels below the lower limit for linear quantitation, the viral load was set at half the lower limit (eg, 50 copies/mL in plasma samples and 50 copies per swab in genital secretion samples). As specified a priori, all analyses included the baseline cervical or vaginal HIV-1 RNA level, to adjust for pretreatment differences. Cofactors associated with genital HIV-1 RNA levels on univariate analysis at $P < .10$ were included in an initial multivariate model. In a second multivariate model, adjustment for reduction in plasma viral load was used to determine the extent to which the effects were independent of the effect of ART on plasma viremia.

Nonparametric comparisons were tested by the Mann-Whitney *U* test. Data were analyzed using SPSS (version 12.0; SPSS).

Results. From February 2005 through January 2008, 102 nonpregnant, HIV-1-seropositive women initiated ART. Baseline characteristics are presented in Table 1. Two women had brief exposure to ART prior to enrollment (1 d of treatment 1 week before enrollment and 3 d of treatment 4 months before enrollment). Of the 102 women who initiated ART, 97 (95.1%) remained in follow-up at month 3 and 95 (93.1%) at month 6. Seven women did not complete the study: 1 discontinued ART and withdrew, 2 died while undergoing treatment for tuberculosis, and 4 were lost to follow-up. Among the women who remained in follow-up, 3 discontinued ART because of adverse drug events, 1 of whom resumed therapy during the study. Six additional women who remained in follow-up had their regimens changed: 4 because of tuberculosis treatment

Table 1. Baseline Characteristics of 102 Women Initiating Antiretroviral Therapy

Variable	No. (%) of participants
Median age, years (IQR)	36 (32–40)
Median education level, years (IQR)	7 (6–9)
Widowed or divorced	74 (72.5)
Works in a bar	64 (62.7)
Contraception other than condoms	
Depot medroxyprogesterone acetate	18 (17.6)
Tubal ligation or hysterectomy	6 (5.9)
Oral contraceptive pills	5 (4.9)
Norplant	5 (4.9)
Sexual risk behavior in past week	
Median no. of partners (IQR)	0 (0–1)
Median no. of times having intercourse (IQR)	0 (0–1)
100% condom use[a]	24 (75.0)
Median CD4 cell count, cells/μL (IQR)	122 (78–164)
WHO clinical stage	
Stage I	18 (17.6)
Stage II	29 (28.4)
Stage III	44 (43.1)
Stage IV	11 (10.8)

NOTE. Data are no. (%) of patients, unless otherwise indicated. IQR, interquartile range; WHO, World Health Organization.

[a] Among the 32 women who were sexually active.

Table 2. Change in Log_{10} HIV-1 RNA Level over 2 Time Periods

Genital secretion sampled, time period, variable	Unadjusted β[a] (95% CI)	*P*	Multivariate 1 β[a] (95% CI)	*P*	Multivariate 2 β[a] (95% CI)	*P*
Cervical						
Months 0–3						
Log_{10} decrease in PVL	−0.368 (−0.476 to −0.261)	<.001	…	…	−0.247 (−0.364 to −0.131)	<.001
Mean percent adherence	−0.037 (−0.049 to −0.024)	<.001	−0.037 (−0.049 to −0.024)	<.001	−0.024 (−0.036 to −0.011)	<.001
Baseline CD4 cell count of <100 cells/μL	0.239 (−0.010 to 0.488)	.059	0.270 (0.053 to 0.488)	.015	0.221 (0.020 to 0.421)	.032
Exposure to hormonal contraception						
DMPA	0.253 (−0.035 to 0.540)	.084	0.276 (0.033 to 0.519)	.026	0.165 (−0.063 to 0.394)	.154
Oral contraceptive pills	−0.199 (−0.662 to 0.265)	.397	−0.053 (−0.446 to 0.339)	.789	−0.062 (−0.423 to 0.298)	.732
Norplant	−0.101 (−0.648 to 0.446)	.715	0.146 (−0.324 to 0.615)	.539	0.018 (−0.417 to 0.453)	.935
Months 0–6[b]						
Log_{10} decrease in PVL	−0.337 (−0.432 to 0.242)	<.001	…	…	−0.210 (−0.316 to −0.103)	<.001
Mean percent adherence	−0.035 (−0.044 to −0.026)	<.001	−0.031 (−0.041 to −0.022)	<.001	−0.025 (−0.034 to −0.015)	<.001
Drug resistance in plasma samples at month 6	0.857 (0.365 to 1.350)	.001	0.376 (−0.073 to 0.824)	.099	0.010 (−0.445 to 0.466)	.964
Baseline CD4 cell count of <100 cells/μL	0.155 (−0.086 to 0.396)	.204	…	…	…	…
Exposure to hormonal contraception						
DMPA	0.140 (−0.136 to 0.416)	.316	…	…	…	…
Oral contraceptive pills	−0.221 (−0.666 to 0.224)	.327	…	…	…	…
Norplant	−0.151 (−0.676 to 0.374)	.569	…	…	…	…
Vaginal						
Months 0–3						
Log_{10} decrease in PVL	−0.030 (−0.043 to −0.018)	<.001	…	…	−0.141 (−0.258 to −0.024)	.019
Mean percent adherence	−0.039 (−0.049 to −0.028)	<.001	−0.038 (−0.049 to −0.028)	<.001	−0.032 (−0.045 to −0.019)	<.001
Baseline CD4 cell count of <100 cells/μL	0.217 (−0.009 to 0.443)	.060	0.200 (0.018 to 0.381)	.031	0.204 (0.014 to 0.393)	.036
Exposure to hormonal contraception						
DMPA	0.081 (−0.183 to 0.345)	.545	…	…	…	…
Oral contraceptive pills	−0.173 (−0.612 to 0.266)	.437	…	…	…	…
Norplant	−0.087 (−0.602 to 0.429)	.739	…	…	…	…
Months 0–6						
Log_{10} decrease in PVL	−0.039 (−0.053 to −0.026)	<.001	…	…	−0.236 (−0.343 to −0.130)	<.001
Mean percent adherence	−0.037 (−0.047 to −0.027)	<.001	−0.033 (−0.044 to −0.023)	<.001	−0.025 (−0.035 to −0.015)	<.001
Drug resistance in plasma samples at month 6	0.999 (0.487 to 1.511)	<.001	0.482 (0.025 to 0.939)	.039	0.108 (−0.341 to 0.558)	.633
Baseline CD4 cell count of <100 cells/μL	0.052 (−0.203 to 0.307)	.688	…	…	…	…
Exposure to hormonal contraception						
DMPA	0.107 (−0.183 to 0.397)	.467	…	…	…	…
Oral contraceptive pills	−0.213 (−0.692 to 0.266)	.380	…	…	…	…
Norplant	−0.237 (−0.801 to 0.328)	.407	…	…	…	…

NOTE. All coefficients are adjusted for baseline genital human immunodeficiency virus type 1 (HIV-1) RNA level. Multivariate model 1 includes all predictors other than plasma viral load (PVL) that were associated with genital HIV-1 RNA levels on univariate analysis at $P < .10$. Multivariate model 2 includes additional adjustment for decrease in PVL. Cervical data were missing for 1 woman due to hysterectomy. CI, confidence interval; DMPA, depot medroxyprogesterone acetate.

[a] Change in the decrease of genital HIV-1 RNA level for each 1-unit change in predictor variable. Negative β values represent greater decreases; positive β values represent smaller decreases.

[b] Both multivariate models for cervical secretion samples over this period were also adjusted for the presence of bacterial vaginosis at the baseline and at month 6, which was associated with increased cervical HIV-1 RNA level in unadjusted analysis ($P < .10$) but not significant in the final models ($P = .062$ in multivariate model 1; $P = .164$ in multivariate model 2).

(nevirapine to abacavir while on rifampin) and 2 because of neuropathy (stavudine to zidovudine). Patient recall was substituted for pill count to determine adherence at 8.4% of visits. At month 6, the median adherence was 98.6% (interquartile range [IQR], 95.4%–99.7%) and the median CD4 cell count increase was 109 cells/μL (IQR, 66–176 cells/μL).

Median HIV-1 RNA levels in plasma samples decreased from 5.54 copies/mL (IQR, 3.62–6.89 copies/mL; 100% detectable) at the baseline to 2.23 copies/mL (IQR, 1.70–6.37 copies/mL; 59.8% detectable) at month 3 and 1.70 copies/mL (IQR, 1.70–6.43 copies/mL; 27.4% detectable) at month 6. In cervical secretion samples, median HIV-1 RNA levels decreased from 4.04

copies per swab (IQR, 1.70–5.69 copies per swab; 96.0% detectable) at the baseline to 1.70 copies per swab (IQR, 1.70–5.66 copies per swab; 12.5% detectable) at month 3 and 1.70 copies per swab (IQR, 1.70–5.05 copies per swab; 13.8% detectable) at month 6. Cervical data were missing for 1 woman because of hysterectomy. In vaginal secretion samples, the median HIV-1 RNA levels decreased from 3.97 copies per swab (IQR, 1.70–5.70 copies per swab; 86.3% detectable) at the baseline to 1.70 copies per swab (IQR, 1.70–5.47 copies per swab; 34.0% detectable) at month 3 and 1.70 copies per swab (IQR, 1.70–5.07 copies per swab; 35.8% detectable) at month 6.

At month 6, among 69 women with undetectable plasma HIV-1 RNA levels, 7 (10.3%) had detectable cervical HIV-1 (range, 121–636 copies/mL) and 22 (31.9%) had detectable vaginal HIV-1 (range, 103–333 copies/mL). In contrast, among 26 women with plasma HIV-1 RNA levels of ⩾100 copies/mL, 6 (23.1%) had detectable cervical HIV-1 (range, 286–111,992 copies per swab) and 12 (46.2%) had detectable vaginal HIV-1 (range, 107–118,325 copies per swab). Genital HIV-1 RNA levels were higher when plasma HIV-1 RNA was detectable ($P = .06$ for cervical secretion samples; $P = .05$ for vaginal secretion samples). Overall, among the 95 women in follow-up at month 6, HIV-1 RNA was detected in secretion samples from both sites for 4 women (4.2%), in cervical secretion samples only for 9 women (9.5%), in vaginal secretion samples only for 30 women (31.6%), and not in secretion samples from either site for 52 women (54.7%).

Among the 95 women who were still in follow-up at month 6, 14 (14.7%) had plasma viral loads of >1000 copies/mL. Sequences could be amplified from plasma samples from 11 women, and genotypic resistance to antiretrovirals was demonstrated in 5 women, of whom 4 had detectable genital HIV-1 shedding at one or both sites. Genotypic resistance was associated with lower adherence (median, 87.7% vs 98.8%; $P = .01$). Plasma resistance mutations included the following, in order of frequency: M184V/I (4 samples), G190A (2 samples), K103N (2 samples), K101E (1 sample), V106A (1 sample), and Y181C (1 sample).

In univariate and adjusted analyses (Table 2), adherence was a strong predictor of cervical HIV-1 suppression at both months 3 and 6 after ART initiation and remained statistically significant after adjustment for plasma viral load. Low pretreatment CD4 cell count and depot medroxyprogesterone acetate use were associated with higher cervical HIV-1 shedding at month 3. Genotypic resistance to antiretrovirals was associated with significantly higher cervical HIV-1 RNA level at month 6, although this association was of marginal significance after adjustment for adherence ($P = .10$) and was eliminated by further adjustment for plasma viral load.

Adherence to ART was also a strong predictor of the magnitude of vaginal HIV-1 suppression at both months 3 and 6 (Table 2). Low pretreatment CD4 cell count was associated with higher vaginal HIV-1 shedding at month 3. Resistance to antiretrovirals in plasma samples was associated with higher vaginal HIV-1 RNA level at month 6 and remained so after adjustment for adherence. This association disappeared after adjustment for plasma viral load.

With the exception of BV (see Table 2), genital ulcers and infections were not associated with genital shedding. Results were similar in all sensitivity analyses.

Discussion. In this large prospective study, adherence was the most important determinant of genital HIV-1 shedding during women's first 6 months of NNRTI-based ART and remained a significant predictor after adjustment for plasma viral load. Genotypic drug resistance in plasma samples was also associated with higher levels of vaginal HIV-1 shedding—an effect that appeared to be mediated through higher plasma viral load. HIV-1 shedding was more common in vaginal than in cervical secretion samples and occurred even in women with suppressed plasma HIV-1 RNA levels. Because drug exposure is the primary mechanism by which adherence effects virus levels, differential drug penetration in the female genital tract may be a cause of this finding [14].

To our knowledge, this is the largest prospective study of female genital HIV-1 shedding after ART initiation that has been conducted to date. We had high participant retention, with over 93% of women remaining in follow-up at month 6. We used an intent-to-treat design, providing a realistic evaluation of female genital tract viral load suppression during the first 6 months of ART. Finally, by evaluating changes in the quantity of HIV-1 RNA rather than detection above an arbitrary limit, our results may be less dependent on an unknown threshold of infectivity.

Although this study focused on women who were eligible for a WHO first-line treatment regimen in Kenya, the results are applicable to a wide range of settings in high-prevalence areas. In addition, although women attending this clinic have a history of transactional sex work, most of the women in the study had low levels of sexual activity at ART initiation. Some contamination of genital secretions by HIV-1 RNA in male ejaculate may have been missed by semen detection; however, such misclassification would be expected to bias results toward the null. We were unable to test levels of genital herpes simplex virus type 2, which may be associated with genital HIV-1 shedding during ART. Other limitations include a relatively short follow-up duration, the possibility that ART administration in a research setting may have led to more favorable outcomes, and uncertainty about the significance of low-level genital HIV-1 shedding.

Higher levels of genital HIV-1 RNA have been associated with an increased risk of heterosexual transmission within discordant couples and appear to be an important surrogate marker for

HIV-1 infectivity [15]. Our results demonstrated a strong and continuous association between ART adherence and genital HIV-1 shedding. Plasma HIV-1 genotypic resistance was associated with both lower adherence and higher levels of genital HIV-1 shedding in women. Optimizing adherence may therefore be important as a means of preventing resistance and maximizing the effect of ART for reducing the risk of HIV-1 transmission.

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