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

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Abstract

Persistent genital HIV-1 shedding among women taking antiretroviral therapy (ART) may present a transmission risk. We investigated associations between genital HIV-1 suppression after ART initiation and adherence, resistance, pre-treatment CD4 count, and hormonal contraceptive use. First-line ART was initiated in 102 women. Plasma and genital HIV-1 RNA were measured at months 0, 3, and 6. Adherence was a strong and consistent predictor of genital HIV-1 suppression ($p<0.001$), while genotypic resistance was associated with higher vaginal HIV-1 RNA at 6 months ($p=0.04$). These results emphasize the importance of adherence to optimize the potential benefits of ART for reducing HIV-1 transmission risk.

Keywords

antiretroviral therapy; HIV infection; women; genital HIV-1 shedding

Introduction

Antiretroviral therapy (ART) has improved health for millions of women living with HIV-1 [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 taking ART [6], including shedding of drug-resistant virus [7], has been documented. Given increasing interest in ART for

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reducing sexual HIV-1 transmission [8], data regarding the prevalence and correlates of genital HIV-1 shedding among treated individuals are needed to inform development of novel prevention interventions.

ART regimens containing non-nucleoside reverse transcriptase inhibitors (NNRTIs) are 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 pre-treatment CD4 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, non-pregnant women were invited to participate if they were eligible for ART according to Kenyan National Guidelines (CD4 cell count ≤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 WHO and Kenyan National Guidelines at the time [10]. During month one, directly administered therapy was used to observe one dose on each weekday. Pill box organizers were used to promote adherence, which was monitored at each visit by pill count ([number of pills taken divided by total number expected] × 100%). If the pill box was unavailable, adherence was calculated using patient recall of pills taken.

Women were screened for genital infections prior to ART initiation. At baseline and monthly thereafter, women were interviewed using standardized questionnaires about sexual behavior, contraceptive practices, and genitourinary symptoms. A pelvic speculum examination was performed, and included collection of specimens for diagnosis of genital infections and viral quantitation, following our published methods [3,11]. If women were menstruating, the examination was rescheduled. Blood was collected for HIV-1 quantitation at baseline and quarterly thereafter.

After Gram staining of cervical secretions, polymorphonuclear leukocytes were counted in three non-adjacent oil immersion fields on microscopy. Yeast and *T. vaginalis* were detected by microscopy of wet preparations. Bacterial vaginosis (BV) was evaluated by Nugent scoring of vaginal Gram stains. Sperm on cervical Gram stain or vaginal wet preparation were noted. Culture for *N. gonorrhoeae* was performed on modified Thayer-Martin medium. Nucleic acid amplification assays (Aptima Combo 2, Gen-Probe, San Diego, California, USA) 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 (FACS Count, Becton Dickinson, Forest Lakes, NJ). Plasma samples were frozen at −70°C until shipment to Seattle for HIV-1 RNA quantitation using the Gen-Probe HIV-1 viral load assay (San Diego, California, USA) [12]. The lower limit of quantitation was 100 copies/swab in genital secretions and 100 copies/ml in plasma. Genotypic resistance was evaluated in plasma samples from month 6 with HIV-1 RNA levels >1,000 copies/mL. A nested real-time polymerase chain reaction (RT-PCR) method designed for HIV-1 subtypes common in

Kenya was used to amplify an ≈800-base pair fragment of reverse transcriptase [13]. Product sequences of two independent RT-PCRs were evaluated; additional RT-PCRs were performed as needed to verify mutations detected in only one of two initial PCRs. The Stanford HIV sequence database (hivdb.stanford.edu) was used to identify drug resistance mutations. If viral cDNA could not be amplified, we assumed that no drug-resistant variants were present.

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

For samples with HIV-1 RNA below the lower limit for linear quantitation, viral load was set at half the lower limit (e.g., 50 copies/mL and 50 copies/swab, respectively). As specified a priori, all analyses included baseline cervical or vaginal HIV-1 RNA, to adjust for pre-treatment differences. Cofactors associated with genital HIV-1 RNA levels on univariate analysis at $p<0.10$ 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 effects were independent of ART's effect on plasma viremia.

Non-parametric comparisons were tested by Mann-Whitney U test. Data were analyzed using SPSS version 12.0 (SPSS, Chicago, Illinois, USA).

Results

Between February 2005 and January 2008, 102 non-pregnant, HIV-1-seropositive women initiated ART. Baseline characteristics are presented in Table 1. Two women had brief exposure to ART (1 day and 3 days) occurring 1 week and 4 months, respectively, prior to enrollment. Of the 102 women initiating 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: one discontinued ART and withdrew, two died while undergoing treatment for TB, and four were lost to follow-up. Among women remaining in follow-up, three discontinued ART due to adverse drug events, one of whom resumed therapy during the study. Six additional women who remained in follow-up had regimen changes: four due to TB treatment (nevirapine to abacavir while on rifampin) and two due to neuropathy (stavudine to zidovudine). Patient recall was substituted for pill count adherence at 8.4% of visits. At month 6, median adherence was 98.6% (IQR, 95.4%–99.7%) and median CD4 count gain was 109 cells/μL (IQR, 66–176 cells/μL).

Median HIV-1 RNA levels in plasma decreased from 5.54 copies/mL (IQR, 3.62–6.89 copies/mL, 100% detectable) at 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 secretions, median HIV-1 RNA levels decreased from 4.04 copies/swab (IQR, 1.70–5.69 copies/swab, 96.0% detectable) at baseline to 1.70 copies/swab (IQR, 1.70–5.66 copies/swab, 12.5% detectable) at month 3 and 1.70 copies/swab (IQR, 1.70–5.05 copies/swab, 13.8% detectable) at month 6. Cervical data were missing for one woman due to hysterectomy. In vaginal secretions, median HIV-1 RNA levels

decreased from 3.97 copies/swab (IQR, 1.70–5.70 copies/swab, 86.3% detectable) at baseline to 1.70 copies/swab (IQR, 1.70–5.47 copies/swab, 34.0% detectable) at month 3 and 1.70 copies/swab (IQR, 1.70–5.07 copies/swab, 35.8% detectable) at month 6.

At month 6, among 69 women with undetectable plasma HIV-1 RNA, 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 ≥100 copies/mL, 6 (23.1%) had detectable cervical HIV-1 (range, 286–111,992 copies/swab) and 12 (46.2%) had detectable vaginal HIV-1 (range, 107–118,325 copies/swab). Genital HIV-1 RNA levels were higher when plasma HIV-1 RNA was detectable (p=0.06 for cervical secretions, p=0.05 for vaginal secretions). Overall, in the 95 women in follow-up at month 6, HIV-1 RNA was detected at both sites for 4 women (4.2%), in cervical secretions only for 9 women (9.5%), in vaginal secretions only for 30 women (31.6%), and at neither site for 52 women (54.7%).

Among the 95 women still in follow-up at month 6, 14 (14.7%) had plasma viral load >1,000 copies/mL. Sequences could be amplified from plasma in 11 cases, and genotypic resistance to antiretrovirals was demonstrated in five, of whom four had detectable genital HIV-1 shedding at one or both sites. Genotypic resistance was associated with lower adherence (median 87.7% versus 98.8%, p=0.01). Plasma resistance mutations included, in order of frequency: M184V/I (4), G190A (2), K103N (2), K101E (1), V106A (1), and Y181C (1).

In univariate and adjusted analyses (Table 2), adherence was a strong predictor of cervical HIV-1 suppression at both 3 and 6 months from ART initiation, remaining statistically significant after adjustment for plasma viral load. Low pre-treatment CD4 count and DMPA 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 at month 6, although this association was of marginal significance after adjustment for adherence (p=0.10) 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 3 and 6 months (Table 2). Low pre-treatment CD4 count was associated with higher vaginal HIV-1 shedding at month 3. Resistance to antiretrovirals in plasma was associated with higher vaginal HIV-1 RNA at month 6, remaining so after adjustment for adherence. This association disappeared after adjustment for plasma viral load.

With the exception of BV (Table 2, footnote b), 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, remaining a significant predictor after adjustment for plasma viral load. Genotypic drug resistance in plasma 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 secretions, and occurred even in women with suppressed plasma HIV-1 RNA. 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 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 on 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.

While this study focused on women eligible for a WHO first-line treatment regimen in Kenya, 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 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 genital HSV-2 levels, 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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Table 1

Baseline characteristics of 102 women initiating ART

Variable	Median (interquartile range) or number (%)
Age (years)	36 (32–40)
Education (years)	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 behaviour in past week	
Number of partners	0 (0–1)
Frequency of intercourse	0 (0–1)
100% condom use[a]	24 (75.0)
CD4 cell count (cells/mm^3)	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)

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

Table 2

Change in $\log_{10}$ HIV-1 RNA level over two time periods[a]

Cervical Secretions	**Unadjusted**		**Multivariate 1 (no PVL)**		**Multivariate 2 (with PVL)**	
Month 0 – Month 3	**Beta (95% CI)**	**P value**	**Beta (95% CI)**	**P value**	**Beta (95% CI)**	**P value**
$\log_{10}$ reduction in plasma viral load	−0.368 (−0.476 to −0.261)	<0.001			−0.247 (−0.364 to −0.131)	<0.001
Mean percent adherence	−0.037 (−0.049 to −0.024)	<0.001	−0.037 (−0.049 to −0.024)	<0.001	−0.024 (−0.036 to −0.011)	<0.001
Baseline CD4 <100 cells/μL	0.239 (−0.010 to 0.488)	0.059	0.270 (0.053 to 0.488)	0.015	0.221 (0.020 to 0.421)	0.032
Exposure to hormonal contraception						
Exposure to DMPA	0.253 (−0.035 to 0.540)	0.084	0.276 (0.033 to 0.519)	0.026	0.165 (−0.063 to 0.394)	0.154
Exposure to oral contraceptive pills	−0.199 (−0.662 to 0.265)	0.397	−0.053 (−0.446 to 0.339)	0.789	−0.062 (−0.423 to 0.298)	0.732
Exposure to Norplant	−0.101 (−0.648 to 0.446)	0.715	0.146 (−0.324 to 0.615)	0.539	0.018 (−0.417 to 0.453)	0.935
Cervical Secretions						
Month 0 – Month 6[b]						
$\log_{10}$ reduction in plasma viral load	−0.337 (−0.432 to 0.242)	<0.001			−0.210 (−0.316 to −0.103)	<0.001
Mean percent adherence	−0.035 (−0.044 to −0.026)	<0.001	−0.031 (−0.041 to −0.022)	<0.001	−0.025 (−0.034 to −0.015)	<0.001
Resistance in plasma at month 6	0.857 (0.365 to 1.350)	0.001	0.376 (−0.073 to 0.824)	0.099	0.010 (−0.445 to 0.466)	0.964
Baseline CD4 <100 cells/μL	0.155 (−0.086 to 0.396)	0.204				
Exposure to hormonal contraception						
Exposure to DMPA	0.140 (−0.136 to 0.416)	0.316				
Exposure to oral contraceptive pills	−0.221 (−0.666 to 0.224)	0.327				
Exposure to Norplant	−0.151 (−0.676 to 0.374)	0.569				
Vaginal Secretions	Unadjusted		Multivariate 1 (no PVL)		Multivariate 2 (with PVL)	
Month 0 – Month 3	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value
$\log_{10}$ reduction in plasma viral load	−0.030 (−0.043 to −0.018)	<0.001			−0.141 (−0.258 to −0.024)	0.019
Mean percent adherence	−0.039 (−0.049 to −0.028)	<0.001	−0.038 (−0.049 to −0.028)	<0.001	−0.032 (−0.045 to −0.019)	<0.001
Baseline CD4 <100 cells/μL	0.217 (−0.009 to 0.443)	0.060	0.200 (0.018 to 0.381)	0.031	0.204 (0.014 to 0.393)	0.036
Exposure to hormonal contraception						
Exposure to DMPA	0.081 (−0.183 to 0.345)	0.545				
Exposure to oral contraceptive pills	−0.173 (−0.612 to 0.266)	0.437				
Exposure to Norplant	−0.087 (−0.602 to 0.429)	0.739				

Cervical Secretions	Unadjusted		Multivariate 1 (no PVL)		Multivariate 2 (with PVL)	
Month 0 – Month 3	**Beta (95% CI)**	**P value**	**Beta (95% CI)**	**P value**	**Beta (95% CI)**	**P value**
Vaginal Secretions						
Month 0 – Month 6						
Log_{10} reduction in plasma viral load	−0.039 (−0.053 to −0.026)	<0.001			−0.236 (−0.343 to −0.130)	<0.001
Mean percent adherence	−0.037 (−0.047 to −0.027)	<0.001	−0.033 (−0.044 to −0.023)	<0.001	−0.025 (−0.035 to −0.015)	<0.001
Resistance in plasma at month 6	0.999 (0.487 to 1.511)	<0.001	0.482 (0.025 to 0.939)	0.039	0.108 (−0.341 to 0.558)	0.633
Baseline CD4 <100 cells/μL	0.052 (−0.203 to 0.307)	0.688				
Exposure to hormonal contraception						
Exposure to DMPA	0.107 (−0.183 to 0.397)	0.467				
Exposure to oral contraceptive pills	−0.213 (−0.692 to 0.266)	0.380				
Exposure to Norplant	−0.237 (−0.801 to 0.328)	0.407				

Beta = Change in reduction of genital HIV-1 RNA for each one-unit change in predictor variable. Negative betas represent greater reduction; positive betas represent smaller reduction.

DMPA = Depot medroxyprogesterone acetate

PVL = plasma viral load

[a] All coefficients adjusted for baseline genital HIV-1 RNA level. Multivariate 1 model includes all predictors other than plasma viral load that were associated with genital HIV-1 RNA levels on univariate analysis at p<0.10. Multivariate 2 model includes additional adjustment for reduction in plasma viral load. Cervical data were missing for one woman due to hysterectomy.

[b] Both multivariate models for cervical secretions over this period were also adjusted for the presence of bacterial vaginosis at baseline and month 6, which was associated with increased cervical HIV-1 RNA level in unadjusted analysis at p < 0.10 but not significant in the final models (p = 0.062 in Multivariate 1, p = 0.164 in Multivariate 2).