Changes in HIV-1 Subtypes B and C Genital Tract RNA in Women and Men After Initiation of Antiretroviral Therapy

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Background. Combination antiretroviral therapy (cART) reduces genital tract human immunodeficiency virus type 1 (HIV-1) load and reduces the risk of sexual transmission, but little is known about the efficacy of cART for decreasing genital tract viral load (GTVL) and differences in sex or HIV-1 subtype.

Methods. HIV-1 RNA from blood plasma, seminal plasma, or cervical wicks was quantified at baseline and at weeks 48 and 96 after entry in a randomized clinical trial of 3 cART regimens.

Results. One hundred fifty-eight men and 170 women from 7 countries were studied (men: 55% subtype B and 45% subtype C; women: 24% subtype B and 76% subtype C). Despite similar baseline CD4$^+$ cell counts and blood plasma viral loads, women with subtype C had the highest GTVL (median, 5.1 log$_{10}$ copies/mL) compared to women with subtype B and men with subtype C or B (4.0, 4.0, and 3.8 log$_{10}$ copies/mL, respectively; $P < .001$). The proportion of participants with a GTVL below the lower limit of quantification (LLQ) at week 48 (90%) and week 96 (90%) was increased compared to baseline (16%; $P < .001$ at both times). Women were significantly less likely to have GTVL below the LLQ compared to men (84% vs 94% at week 48, $P = .006$; 84% vs 97% at week 96, $P = .002$), despite a more sensitive assay for seminal plasma than for cervical wicks. No difference in GTVL response across the 3 cART regimens was detected.

Conclusions. The female genital tract may serve as a reservoir of persistent HIV-1 replication during cART and affect the use of cART to prevent sexual and perinatal transmission of HIV-1.

Keywords. HIV-1 genital tract RNA; HIV-1 subtypes B and C; antiretroviral drugs.

Blood plasma and genital tract human immunodeficiency virus type 1 (HIV-1) RNA concentrations are predictors of risk for heterosexual [1, 2] and perinatal HIV-1 transmission [3–5], and differences in blood plasma viral load (BPVL) between men and women

with similar CD4$^+$ cell counts have been observed [6–11]. It is unknown whether there are sex-related differences in genital tract viral load (GTVL), because few clinical trials in which GTVL has been measured have enrolled sufficient numbers of both men and women. Some studies have suggested, however, that women are less likely to durably suppress GTVL compared to men [12, 13]. This may be related to known differences in antiretroviral (ART) penetration found between men and women and among different specific ART drugs [14, 15] or to differences in coinfections or the immunologic milieu between men and women (reviewed in [16]). Blood and seminal plasma (SP) viral loads (VLs) may be higher with HIV-1 subtype C infection

compared with other subtypes [17–20] and might, in part, explain the rapid spread of HIV-1 in areas of the world where subtype C predominates. The effect of antiretroviral therapy on GTVL of different HIV-1 subtypes is unknown.

MATERIALS AND PARTICIPANTS

The AIDS Clinical Trials Group (ACTG) study A5185s was a substudy of the A5175, PEARLS Trial (Prospective Evaluation of Anti-retroviral Combinations for Treatment Naive, HIV Infected Persons in Resource-Limited Settings; ClinicalTrials.gov NCT00084136) [21]. Antiretroviral-naive participants with $CD4^+$ lymphocytes <300/µL were randomly assigned (1:1:1) to 1 of 3 open-label combination antiretroviral (cART) regimens: efavirenz plus co-formulated lamivudine-zidovudine (EFV + 3TC/ZDV); or atazanavir plus didanosine EC plus emtricitabine (ATV + DDI + FTC); or efavirenz plus co-formulated emtricitabine-tenofovir DF (EFV + FTC/TDF). All participants meeting the entry criteria for PEARLS at participating sites were offered enrollment to A5185s and provided separate informed consent. A5185s was designed to evaluate differences in GTVL between men and women and between HIV-1 subtypes B and C, as well as the effect of cART on GTVL.

Specimen Collection and Processing
Genital secretions and blood plasma specimens were obtained at study entry and at weeks 48 and 96. For men, semen was collected at the clinic or within 2 hours prior to the clinic visit by masturbation following a prescribed abstinence of 48 hours. Following liquefaction, the total semen volume was determined and the sample centrifuged at 600g for 15 minutes. For women, a pelvic examination was performed with collection of 2 endocervical wicks (Tear-Flo Diagnostic Test Strip, Odyssey, Bartlett, TN). Each wick was cut at the "15" mark and the 2 distal round ends, which delivered 12 µL each, were placed into a 2-mL cryovial containing 500 µL of NASBA Buffer solution (bioMérieux, Durham, NC) and stored at −70°C. Women refrained from any kind of sexual activity, douching, or insertion of intravaginal products for at least 48 hours prior to the collection of cervical specimens. Genital specimens were processed within 4 hours of collection and stored at −70°C. Specimens were shipped to the University of Washington ACTG Virology Specialty Laboratory, Seattle, for HIV-1 RNA quantification.

Laboratory Testing
Blood plasma HIV-1 RNA and $CD4^+$ cell counts were measured in PEARLS [21]. HIV-1 RNA was extracted from SP or cervical wicks (CWs) and quantified using an independently validated in-house real-time polymerase chain reaction assay, with a lower limit of quantification (LLQ) of 120 copies/mL (SP) and 1575 copies/mL (CW) and an upper limit of quantification

of 5 000 000 (SP) and 52 500 000 copies/mL (CW). The difference in the LLQ between SP and CW was due to the requirement for sample dilution during specimen processing. We did not control for HIV-1 DNA; the assay precision was <0.24 $\log_{10}$ nucleic acid copies/mL for genital specimens from men and women [22, 23].

HIV-1 subtype was determined by DNA sequence analysis of reverse transcriptase and protease in 103 participants who were also included in a case-cohort study of virologic failures in the main study (S. Eshleman, unpublished observations). Of these, 29 had HIV-1 subtype B, 71 had C, 2 had F1, and 1 had R12. All 71 participants infected with subtype C were from a subtype C–dominant country, whereas 91% (29/32) of participants from a subtype B–dominant country were infected with subtype B. For the remaining 228 participants in the A5185s substudy, HIV-1 subtype was imputed using the dominant subtype in the country where the participant was enrolled. Overall, this resulted in 128 participants with subtype B, 200 with subtype C, and 3 with neither subtype C or B; the latter 3 were excluded from all analyses.

Statistical Analyses
Baseline characteristics were compared among the 4 sex/subtype groups using the Wilcoxon rank-sum test for continuous variables and the χ^2 test for categorical variables. The Spearman correlation was used to measure the association between GTVL and BPVL. A median regression model was used to compare baseline GTVL among sex/subtype groups by including main effects for sex and subtype as well as an interaction term for sex and subtype. The model was extended by including BPVL as a covariate to assess the association between GTVL and BPVL, and by also adding age and presence of a sexually transmitted infection (STI) to evaluate whether the interaction of sex and subtype persisted when adjusted for BPVL, age, and proportion with an STI. Change in the proportion of women with VL below the LLQ from baseline to weeks 48 and 96 was evaluated using the McNemar test. Logistic regression was used to evaluate differences according to sex/subtype and among randomized treatments at weeks 48 and 96 in the proportion of participants with GTVL below the LLQ.

RESULTS

Baseline Characteristics
Between May 2005 and November 2006, 328 individuals (158 men and 170 women; 128 infected with HIV subtype B and 200 with subtype C) of the 1571 (21%) total PEARLS participants enrolled in A5185s and provided baseline genital secretions. Participants were from 16 sites in 7 countries. The distribution of men and women varied across countries. Enrollment in Brazil, India, Peru, and the United States included

Table 1. Selected Baseline Characteristics by Sex and HIV-1 Subtype

Characteristic	Male Subtype B (n = 87)	Male Subtype C (n = 71)	Female Subtype B (n = 41)	Female Subtype C (n = 129)	Total (N = 328)	P Value
Age, y						
Median	34	34	33	32	33	.0682[a]
Q1, Q3	29, 42	30, 40	27, 40	27, 37	28, 39	
Country						
Brazil	40 (46%)	0 (0%)	12 (29%)	0 (0%)	52 (16%)	<.0001[b]
India	0 (0%)	19 (27%)	0 (0%)	9 (7%)	28 (9%)	
Malawi	0 (0%)	36 (51%)	0 (0%)	82 (64%)	118 (36%)	
Peru	26 (30%)	0 (0%)	19 (46%)	0 (0%)	45 (14%)	
South Africa	0 (0%)	11 (15%)	0 (0%)	27 (21%)	38 (12%)	
United States	21 (24%)	0 (0%)	10 (24%)	0 (0%)	31 (9%)	
Zimbabwe	0 (0%)	5 (7%)	0 (0%)	11 (9%)	16 (5%)	
Screening CD4$^+$ count, cells/mm^3						
Median	196	189	171	193	193	.9002[a]
Q1, Q3	90, 259	121, 248	121, 234	133, 237	123, 243	
Plasma HIV-1 viral load, log$_{10}$ copies/mL						
Median	5.0	5.0	4.9	4.9	4.9	.1722[a]
Q1, Q3	4.5, 5.4	4.6, 5.5	4.5, 5.3	4.5, 5.2	4.5, 5.3	
Genital secretion HIV-1 viral load, log$_{10}$ copies/mL						
Median	3.8	4.0	4.0	5.1	4.4	<.0001[a]
Q1, Q3	2.7, 4.7	3.0, 4.8	3.2, 4.9	4.4, 5.5	3.4, 5.2	
Sexually transmitted infection						
Yes	30 (34%)	0 (0%)	7 (17%)	4 (3%)	41 (13%)	<.0001[b]
No	57 (66%)	71 (100%)	34 (83%)	125 (97%)	287 (88%)	
Randomized treatment group						
ZDV/3TC + EFV	21 (24%)	26 (37%)	12 (29%)	42 (33%)	101 (31%)	.3166[b]
DDI + FTC + ATV	30 (34%)	26 (37%)	18 (44%)	40 (31%)	114 (35%)	
TDF/FTC + EFV	36 (41%)	19 (27%)	11 (27%)	47 (36%)	113 (34%)	

Abbreviations: 3TC, lamivudine; ATV, atazanavir; DDI, didanosine; EFV, efavirenz; FTC, emtricitabine; HIV-1, human immunodeficiency virus type 1; TDF, tenofovir; ZDV, zidovudine.

[a] Kruskal-Wallis test.

[b] χ^2 test.

more men than women, whereas enrollment in the African countries included more women than men (Table 1). Thus, men were predominantly enrolled in countries in which the dominant subtype is B and women in countries in which the dominant subtype is C. Among men, 87 (55%) had subtype B and 71 (45%) had subtype C, whereas among women, 41 (24%) had subtype B and 129 (76%) had subtype C (Table 1). Selected baseline characteristics of participants for each subtype by sex are shown in Table 1.

The median age for participants in the substudy was 33 years (25th–75th percentiles, 28–39 years; Table 1), varying between 32 and 33 years for women with subtypes C and B, to 34 for
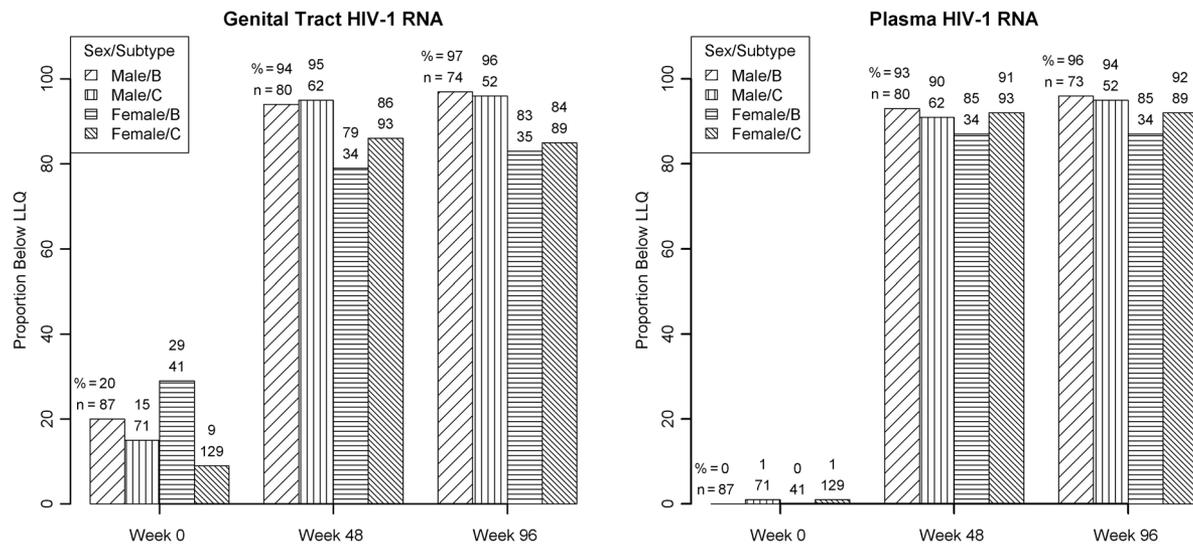
Figure 1. Proportion of participants with genital tract and blood plasma human immunodeficiency virus type 1 (HIV-1) RNA levels below the assay lower limit of quantification by weeks after starting antiretroviral therapy by sex/HIV-1 subtype. Abbreviations: HIV-1, human immunodeficiency virus type 1; LLQ, lower limit of quantification.

men with either subtype ($P = .068$). The median screening CD4+ cell count was 193 cells/µL (25th–75th percentiles, 123–243 cells/µL; Table 1) with no significant difference among the 4 sex/subtype groups ($P = .90$). The median baseline blood plasma HIV-1 RNA was 4.9 (25th–75th percentiles, 4.5–5.3) $\log_{10}$ copies/mL, also with no significant difference among the 4 sex/subtype groups ($P = .17$).

The proportion of participants with STIs reported at baseline also varied significantly among the 4 sex/subtype groups ($P < .0001$), reflecting higher prevalence among participants with subtype B (34% for men and 17% for women) than subtype C (0% for men and 3% for women). Among the 328 participants with complete baseline GTVL, there were 50 STIs reported among 41 participants (30 males with subtype B, 7 females with subtype B, and 4 females with subtype C). Of these 50 events, 19 were syphilis, 15 were genital warts, 11 were gonorrhea, 2 were trichomoniasis, 2 were unspecified venereal disease, and 1 was chlamydial infection. There was no significant difference in baseline GTVL between those with and without STIs ($P = .107$). These results, however, should be interpreted with caution given the small number of female participants with STIs.

Baseline GTVL by Subtype and Sex

HIV-1 RNA was detected in 99% of baseline blood samples, but in only 82% of SP samples and 86% of CW samples. The correlation of baseline BPVL and GTVL was 0.38 for men and 0.27 for women ($P < .001$ for both). There was no evidence that the association between GTVL and BPVL varied by sex or subtype; in regression analysis, median GTVL increased by

0.58 $\log_{10}$ copies/mL for each 1 $\log_{10}$ copies/mL increase in BPVL (95% confidence interval [CI], .38–.77 $\log_{10}$ copies/mL).

There was a significant difference in GTVL among the 4 sex/subtype groups ($P < .001$; Table 1). Women with subtype C had the highest median GTVL (5.1 $\log_{10}$ copies/mL) whereas women with subtype B and men with subtype C or B had similar median GTVL (4.0, 4.0, and 3.8 $\log_{10}$ copies/mL, respectively). The difference in median GTVL between men and women varied significantly according to subtype (sex by subtype interaction $P = .030$). The estimated difference between men and women with subtype B was not significant (difference in medians: 0.27 $\log_{10}$ copies/mL higher for women; 95% CI, −.34 to .88; $P = .38$), whereas there was a significant difference between men and women with subtype C (1.03 $\log_{10}$ copies/mL higher for women; 95% CI, .77–1.29; $P < .001$). The higher GTVL for subtype C was not driven by one particular country: the median GTVL for men and for women with subtype C was 4.0 and 5.1 $\log_{10}$ copies/mL in Malawi, 4.0 and 5.0 $\log_{10}$ copies/mL in South Africa, 4.1 and 5.4 $\log_{10}$ copies/mL in India, and 4.8 and 5.0 $\log_{10}$ copies/mL in Zimbabwe. The difference in median GTVL according to sex/subtype persisted in analysis adjusted by BPVL, age, and STI at baseline (interaction $P = .037$), and so was not explained by the observed differences in BPVL, age, and proportion with an STI according to sex/subtype.

Effect of cART on GTVL

Among the 328 participants with baseline GTVL measurements, 40 (12%; 17 men and 23 women) were lost to follow-up or died before 96 weeks of follow-up. A total of 269 participants (82%;

142 men and 127 women) had evaluable results at week 48, and 250 participants (76%; 126 men and 124 women) had results at week 96. Two hundred twenty-nine participants (70%; 122 men and 107 women) had results at all 3 time points.

Overall, 52 (16%) of the substudy participants had GTVL below the assay LLQ (120 copies/mL for men and 1575 copies/mL for women) at baseline. Of the 87 and 71 men with subtypes B and C, 17 (20%) and 11 (15%) had a baseline GTVL below the LLQ ($P = .51$), and the corresponding figures for the 41 and 129 women with subtypes B and C were 12 (29%) and 12 (9%), respectively ($P = .001$; Figure 1).

The proportion of participants with a GTVL below the LLQ at week 48 (90%) and week 96 (90%) was significantly increased compared to baseline ($P < .001$ at both times). However, women were significantly less likely than men to have GTVL below the LLQ at both times despite the fact that the LLQ for CW samples was >10 times higher than that for SP samples. There was no evidence that the difference between men and women varied by subtype (interaction $P = .83$ and $P = .68$ at weeks 48 and 96, respectively). Specifically, at week 48, 84% of women and 94% of men had GTVL below the LLQ ($P = .006$ comparing women to men, adjusted for subtype; Figure 1). The corresponding proportions at week 96 were 84% and 97% ($P = .002$). In contrast, only 2 (1%) had a BPVL below the assay LLQ (400 copies/mL) at baseline. For weeks 48 and 96, 91% and 93%, respectively, of participants had BPVL below the LLQ with similar results in each of the 4 sex/subtype groups (Figure 1). There was no significant difference among the 3 treatment groups in the proportion of participants with GTVL below the LLQ at week 48 ($P = .57$) or week 96 ($P = .73$) (data not shown).

Effect of Hormonal Contraception

Information on hormonal contraception was available for 168 women. At baseline, 30 women (18%) had been on hormonal contraception for at least 14 days, primarily on medroxyprogesterone (73%). The median GTVL among these women was 5.0 log$_{10}$ copies/mL compared with 4.9 log$_{10}$ copies/mL among women not on hormonal contraception or on hormonal contraception for <14 days ($P = .49$). There was no evidence that the difference between these 2 groups depended on HIV-1 subtype (interaction $P = .30$). There were also no significant differences in median age, baseline CD4 count, or baseline BPVL between these 2 groups. At weeks 48 and 96, the proportion of women on hormonal contraception had increased to 63% and 69%, respectively, with about three-quarters of these on medroxyprogesterone. At weeks 48 and 96, 84% (67 of 80 and 71 of 85, respectively) of women on hormonal contraception had GTVL less than the LLQ compared with 85% (39 of 46 and 33 of 39, respectively) not on hormonal contraception ($P = .88$ at both times).

DISCUSSION

This study demonstrates that despite similar BPVL between men and women and between HIV subtypes B and C, GTVLs were significantly higher at baseline in women infected with subtype C compared to women infected with subtype B and men infected with either subtype. The relationship between GTVL and subtype among women was not associated with hormonal contraception use or STIs, and women with either subtype were significantly less likely to have GTVL below the LLQ at weeks 48 and 96.

In a study of early HIV infection in 188 women, Morrison et al [19] found that Zimbabwean (subtype C) infection was associated with increased cervical VLs compared to Ugandan women infected with subtypes A or C. Women infected with subtype D also had increased cervical VLs, but this was not significant [19]. However, VLs in these women were much lower (mean cervical HIV RNA = 1.64 log$_{10}$ copies/mL) compared to those observed in this study (mean female GTVL = 4.79 log$_{10}$ copies/mL).

In a large study of heterosexual HIV-1–serodiscordant couples from 7 African countries (n = 2521) with median CD4$^+$ cell count of 496, median endocervical VLs of 3.20 log$_{10}$ copies/mL (n = 1805) and 2.57 log$_{10}$ copies/mL in SP (n = 716) were reported by Baeten et al [24]. These values are much lower than those observed in our study (median female GTVL, 4.92 log$_{10}$ copies/mL; median SP VL, 3.91 log$_{10}$ copies/mL). Additionally, although the same laboratory tested genital secretions from both Baeten et al and this study, sampling techniques for the women differed.

Antiretrovirals were very effective in decreasing VLs in both compartments in all participants. At weeks 48 and 96, 89% and 90%, respectively, of all participants had BPVLs that were below the limit of quantification. However, in this study, men were significantly more apt to have a GTVL that was below the limit of quantitation, regardless of subtype and in spite of the difference in the LLQ of the assay for SP (120 copies/mL) compared to the female genital tract (1575 copies/mL). Similar results have been reported previously [12, 13]. Differences in genital tract drug penetration between men and women [14, 15] might explain this finding. Of the drugs used in the study, TDF, 3TC, FTC, DDI, and ZDV have higher seminal plasma-to-blood plasma ratios whereas levels of EFV and ATV are negligible. In women, FTC, 3TC, and ZDV accumulate in the genital tract, whereas ATV and EFV have lower levels in the genital tract than in blood. Blood plasma to cervical secretion ratios for DDI and TDF have varied [15]. The 3 randomized treatment groups had comparable proportions of participants with VLs that were below the limit of quantification both at weeks 48 and 96.

Recently, Cohen et al [25] reported that early initiation of ART reduces the rate of sexual transmission in people with a CD4$^+$ cell count between 350 and 550 cells/μL. Baeten et al [24]

observed that genital HIV RNA independently predicted transmission after adjusting for BPVL. In the absence of ART, 59%–67% of the genetically linked transmissions in these 2 studies have been from women to men. Because participants in our study were more immunocompromised (median CD4$^+$ cell count, 194) and GTVL was 1.3–1.7 $\log_{10}$ copies/mL greater than those in the Baeten et al study, the risks for sexual transmission are presumably greater in the absence of cART.

The strengths of the present study are the relatively large number of participants from ethnically and geographically diverse areas of the world infected with different HIV-1 subtypes. These locations coincide with all but 2 of the sites where PEARLS was conducted and with 6 of 13 HPTN 052 sites [25]. However, A5185s study participants had <300 CD4 cells/μL at entry and are thus more representative of individuals starting antiretrovirals under current World Health Organization treatment guidelines. The same laboratory performed the genital tract HIV RNA assays for the Partners in Prevention HSV/HIV Transmission Study [25], minimizing one potential confounder when trying to make comparisons between studies.

Our study has several important limitations. Participation in A5185s was voluntary and, as a consequence, there may be some selection bias. Assignment of subtype for 69% of the participants was based on country of residence, and there may have been some misclassification. However, we think the likelihood of misclassification is small, as all participating countries except Brazil have a dominant subtype comprising 96%–100% of the sequences for that country [26]. Additionally, there was a nonrandom distribution of HIV subtypes between men and women, and the differences observed were largely driven by the higher GTVL observed in women infected with subtype C from all countries. There were differences in the lower limit of detection of the GTVL assays, although that does not explain the significantly higher GTVL observed in women. We did not measure or control for HIV proviral DNA, which might have contributed to the higher GTVL found in women. Although GTVL can be affected by STIs and bacterial vaginosis, few STIs were recorded and information on bacterial vaginosis was not collected. Unmeasured coinfections and inflammation in women may have led to higher GTVL measurements.

In summary, we have demonstrated that GTVL differs according to sex and HIV subtype, with higher levels in women with subtype C virus. Moreover, women are less likely to suppress GTVL after initiating cART. The female genital tract may serve as a reservoir of persistent HIV-1 replication during cART and affect the use of cART to prevent sexual and perinatal transmission of HIV-1.

Notes

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