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Associations between genital tract infections, genital tract inflammation and cervical cytobrush HIV-1 DNA in US versus Kenyan women

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Abstract

Cervical shedding of HIV-1 DNA may influence HIV-1 sexual transmission. HIV-1 DNA was detected in 250/316 (80%) and 207/259 (79%) cervical cytobrush specimens from 56 United States (US) and 80 Kenyan women, respectively. Plasma HIV-1 RNA concentration was associated with increased HIV-1 DNA shedding among US and Kenyan women. Kenyan women had higher cervicovaginal concentrations of pro-inflammatory interleukins (IL)-1β, IL-6, IL-8 and anti-inflammatory secretory leukocyte protease inhibitor (SLPI) compared to US women (all $p < 0.01$). HIV-1 DNA shedding was associated with increased concentrations of IL-1β and IL-6 and lower SLPI among US women, but not Kenyan women.

Background

Cervical HIV-1 DNA shedding is frequently detected among HIV-1-infected women (26-86% of specimens)[1-3] despite potent suppressive antiretroviral therapy (ART) and may be a contributor to sexual HIV-1 transmission. The mechanisms responsible for HIV-1 DNA

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shedding are not well understood; elucidation of these mechanisms could be useful for prevention of sexual as well as perinatal HIV-1 transmission.

We hypothesized that pro-inflammatory cytokines and co-infections in the female genital tract are associated with cervical HIV-1 DNA shedding. To test this hypothesis, we evaluated associations between vaginal pro-inflammatory cytokines, antiretroviral therapy, vaginal co-infections and HIV-1 DNA shedding in cervical cytobrush samples from HIV-1 infected women in the US and in Kenya.

Methods

Specimens and clinical data were collected during prospective observational studies of HIV-1 infected women in Seattle, WA, Rochester, NY and Nairobi, Kenya between 2002-2009. Study participants were 18-50 years of age, non-pregnant and had no symptomatic genital infections at study entry. The University of Washington, University of Rochester and University of Nairobi Institutional Review Boards approved the studies, and all subjects provided written informed consent.

Participants had 3-4 study visits per year over 1-5 years, as described previously.[4] HIV-1 RNA from plasma and cervicovaginal lavage (CVL) specimens were quantified by real-time PCR assay.[5] HIV-1 DNA was quantified in cervical cytobrush specimens by quantitative PCR for the *gag* region of HIV-1.[6] Samples with 1 to 4 copies of HIV-1 DNA/10uL extracted DNA were reported as <5 copies/cytobrush. The human betaglobin gene[7] was amplified to evaluate the number of cells; samples containing fewer than 100,000 cells were considered inadequate. CVL was tested for interleukins (IL)-1β, -6, -8 and secretory leukocyte protease inhibitor (SLPI) as previously described.[8] Urine was collected for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* detection by nucleic acid amplification (COBAS Amplicor PCR, Roche, Pleasanton, CA). *Trichomonas vaginalis* (TV) was detected by the InPouch culture system (Hardy Diagnostics, Santa Maria, California); testing for trichomoniasis was not performed routinely in Kenyan women, and was excluded from analysis in this group. Genital shedding of herpes simplex virus (HSV) 1/2 and cytomegalovirus (CMV) was evaluated using PCR of CVL.[9, 10] Bacterial vaginosis (BV) was diagnosed using Nugent's criteria.[11] Vaginal yeast and *Lactobacillus* were detected by culture.[12] Detection of an infectious agent in <1% of the genital specimens was considered too sparse for further analysis.

Demographic data at enrollment were compared between US and Kenyan women using Student's t-test and chi-square test. HIV-1 DNA concentrations were grouped for analysis in 4 categories: undetectable, very low (<5 copies/cytobrush), below the median (5-100 copies/cytobrush) and above the median (>100 copies/cytobrush). We used multinomial logistic regression and linear regression with generalized estimating equations specifying an independent correlation structure and robust standard errors to assess associations between genital tract infections, cytokines and HIV-1 DNA shedding. Based on substantial differences in several predictors between US and Kenyan women, these two cohorts of women were analyzed separately. All analyses were conducted using Stata version 10 (StataCorp, Inc., College Station, TX).

Results

Data from 136 women were included: 56 from the US and 80 from Kenya. US women contributed 316 visits with complete data (median 4/woman, interquartile range [IQR] 2-6), and Kenyan women 259 visits (median 3/woman; IQR 2-4). At enrollment, mean age of US participants was 39 ± 6 years compared to 32 ± 5 years for Kenyan women ($p < 0.01$). US

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women were primarily African American (58%), 20% white and 22% other ethnicities. In US women, mean CD4+ T cell count at study entry was 436 ± 258 cells/mL and mean plasma HIV-1 RNA concentration 2.9 ± 1.4 $\log_{10}$ copies/mL, while Kenyan women had both higher CD4+ T cell count (582 ± 232 cells/mL; $p < 0.001$) and plasma HIV-1 RNA concentration (3.4 ± 1.3 $\log_{10}$ copies/mL; $p = 0.004$). ART use was reported by 38/56 (68%) US women at 179/316 visits (57%), in contrast to 8/80 (10%) Kenyan women taking ART at 13/259 visits (5%; $p< 0.01$). When reporting use of ART, US women showed viral suppression (plasma HIV-1 RNA < 30c/mL) at 104/179 (58%) visits, while Kenyan women were suppressed at only 2/13 (15%) visits.

HIV-1 DNA was detected in cervical cytobrush specimens collected at 250 visits from US women (80%) and 207 visits from Kenyan women (79%; $p= 0.57$). Of 139 specimens with undetectable HIV-1 DNA, 21 (11 from US and 10 from Kenyans) had inadequate sample and were excluded, leaving 118 (20%) cervical specimens with undetectable HIV-1 DNA. In women with quantifiable HIV-1 DNA, median quantity of cervical HIV-1 DNA differed by nationality: 25 copies/cytobrush (IQR 5, 91) in US women versus 69 copies/cytobrush (IQR 12, 210) in Kenyan women ($p < 0.01$). However, after adjustment for HIV-1 plasma RNA concentration, this difference was no longer significant ($p = 0.37$). HIV-1 RNA was detected in CVL collected at 59(19%) visits by US women and at 148 (57%); visits by Kenyan women ($p<0.01$). This difference remained significant after controlling for plasma viral load ($p < 0.01$). Quantification of the betaglobin gene detected a median of 640,000 cells/specimen (IQR 321,000, 1,266,700) in US women, higher than the median of 459,000 cells/specimen in Kenyan women (IQR 228,300, 780,000; $p= 0.03$).

US women had significantly lower genital cytokine concentrations compared to Kenyan women. Among US subjects, median IL-1β was 13.7 pg/mL (IQR 3.6, 52.5), compared with 64.9 pg/mL in Kenyan women (IQR 21.2, 264.4) ($p< 0.01$), with significant differences also seen between the median IL-6 in US women of 4.8 pg/mL (IQR 2.5, 16.2) versus 25.5 pg/mL (IQR 9.6, 72.3) in Kenyan women ($p< 0.01$); IL-8, with US median of 245 pg/mL (IQR 114, 635) versus Kenyan median of 1273 pg/mL (IQR 506, 3080; $p< 0.01$); and a median SLPI in US women of 74,559 pg/mL (IQR 21,171, 184,854) versus 222,776 pg/mL (IQR 101,821, 485,043) in Kenyan women ($p< 0.01$). These differences remained significant even after controlling for differences in prevalence of genital tract infections (data not shown).

Women with higher plasma viral load and lower CD4+ T cell count were more likely to have higher quantities of HIV-1 DNA detected in cervical specimens in both US and Kenyan populations (Table 1). In US women, none of the studied genital infections were associated with higher levels of cervical HIV-1 DNA, while in Kenyan women yeast vaginitis was positively and cervicitis negatively associated with cervical HIV-1 DNA shedding. BV and abnormal vaginal flora were associated with increased concentrations of IL-1β in both US and Kenyan women, but other associations differed between women from the two countries (Table 2). Yeast and BV were more common among US women, while cervicitis, CMV and HSV shedding were more common among Kenyan women. Gonorrhea and Chlamydia were present in < 1% of samples, thus were not included in analysis. ART was not associated with HIV-1 DNA shedding in univariate analysis, but when the model was controlled for plasma HIV-1 concentration US women on ART had a higher risk of shedding. We performed a stratified analysis in this group and found that the increased risk of HIV-1 DNA shedding was largely due to the 58% of women who were on ART but not suppressed (data not shown). Too few Kenyan women were on ART to perform the same analysis.

Among US women, after controlling for plasma HIV-1 RNA, the concentration of IL-1β in CVL was significantly higher in women with > 100 copies of HIV-1 DNA in cervical

secretions than those with no HIV-1 DNA shedding (median 47 pg/mL vs 7 pg/mL; p = 0.02). This was also true for IL-6 (median 9 pg/mL vs. 4 pg/mL; p = 0.05) and lower concentrations of SLPI (median 31,119 pg/mL vs. 99,175 pg/mL; p = 0.008). IL-8 was not significantly different between woman with > 100 copies/cytobrush of HIV-1 DNA detected and those with no detectable cervical HIV-1 DNA (median 211 pg/mL vs 245 pg/mL; p = 0.53). In Kenyan women, no differences in cytokine concentrations were seen between specimens with > 100 copies/cytobrush HIV-1 DNA (n = 88) and those with no HIV-1 DNA detected (n = 52) (data not shown).

Discussion

This study of HIV-1 infected Kenyan and US women found higher levels of pro-inflammatory cytokines in the CVL of Kenyan compared to US HIV-1 infected women. Kenyan women had higher rates of some genital infections, such as cervicitis and CMV, but US women were more likely to have yeast. Differences in cytokines persisted after controlling for genital infections, suggesting a larger difference in immune milieu between US and Kenyan women. Cohen et al found that HIV-1 uninfected Kenyan adolescents had a higher number of activated CD4+ T cells in the genital tract than American adolescents[13] even after controlling for genital infections. The genital inflammation in these Kenyan populations may stem from systemic infections with parasites, malaria or TB, from vaginal washing practices, or from unmeasured genital tract infections.

In the US cohort, women with the highest quantities of cervical HIV-1 DNA had higher genital IL-1β and IL-6 concentrations compared to women with no HIV-1 DNA detected. Both cytokines are common to many immune response pathways in the innate and adaptive immune response, thus may implicate activation of any of several pathways and do not point to a specific mechanistic connection.[14-16] Interestingly, no genital infections were positively associated with the highest levels of HIV-1 DNA in either population. Together, these results suggest that there is not a direct causal pathway between the genital infections we studied, these classic pro-inflammatory cytokines and HIV-1 DNA genital shedding. The stimuli for HIV-1 DNA genital shedding may be from infections that were not measured in this cohort, from systemic factors, or from non-infectious local stimuli.

An association between genital infections and cytokine concentrations in genital secretions has been reported across multiple studies, although the cytokines and infections studied have varied. Across studies, BV is associated with IL-1β in both US[17, 18] and Kenyan[19] women, but few reports examined IL-1β and other infections. IL-6 appears to have little relationship with any genital infection[17, 20-22] , while increased IL-8 has been associated with trichomoniasis,[23] cervicitis,[24] and yeast[25] in many, but not all[17, 25] studies. Few of these studies enrolled African women, thus we are unable to assess whether the differences we observed in associations between US and Kenyan women are an anomaly, or reflect regional differences. Even fewer studies have assessed the link between genital infections and genital tract HIV-1 DNA; one showed no association between yeast vulvovaginitis and cervical HIV-1 DNA detection,[2] while a Kenyan study found an association with mucopurulent cervical discharge, but did not assess specific pathogens.[26]

HIV-infected cells may pose a risk for sexual transmission,[27] similar to the association between HIV-1 DNA in blood and breast milk and mother to child transmission.[28] Plasma HIV-1 RNA concentration is the factor most commonly associated with detection of HIV-1 DNA in cervicovaginal secretions.[2, 29, 30] While previous studies have reported lower rates of HIV-1 DNA shedding in women on ART[31-33] compared to those who are not,[1, 10, 30] detection of HIV-1 DNA is much more common than HIV-1 RNA when women have a suppressed plasma viral load.[5] In this study, US women on ART but not suppressed in the

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plasma had a higher risk of HIV-1 DNA shedding than women not on ART. This is likely because in our US cohort women on ART were likely to be sicker (as evidenced by the low rate of plasma viral suppression), creating a more inflammatory environment.

Our study only assessed cervical HIV-1 DNA shedding and not detection in vaginal secretions. Other investigators have found a higher prevalence of HIV-1 DNA shedding in cervical compared to vaginal samples.[26] Our evaluation of cervical cytobrush samples detected HIV-1 DNA at higher rates than studies which have used cervical Dacron swabs or cervicovaginal lavage [1, 29, 30], but similar to rates reported by another study using cytobrush samples.[3] This is likely because the cytobrush picks up more cellular material than swabs or lavage. However, our cytokine measurements were made on CVL fluid, an indirect reflection of cervical inflammation. Our participants contributed visits both on and off ART, and with and without virologic suppression, which increases the heterogeneity of the data and may mask differences in determinants of genital shedding.

Our study did not detect a direct causal pathway linking genital infections, the classic pro-inflammatory cytokines IL-1β, IL-8 and IL-6, and HIV-1 DNA shedding. Additionally, local effects of inflammation or infection on cervical HIV-1 DNA shedding are eclipsed by systemic factors such as plasma HIV-1 RNA concentration.

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Table 1

Evaluation of demographic factors and genital infections for association with cervical HIV-1 DNA shedding, stratified by nationality. Results of multinomial logistic regression analysis comparing each group to women with no HIV-1 DNA shedding detected are reported as Relative Risk Ratio (RRR) (95% CI).

Factor	Not detected		< 5 copies HIV-1 DNA/cytobrush		5-100 copies HIV-1 DNA/cytobrush		100 copies HIV-1 DNA/cytobrush	
US								
# of visits	66		90		103		57	
Log_{10} plasma HIV-1 RNA	2.2 ± 0.9 (Ref)		2.8 ± 1.3 (p = 0.008)*		3.0 ± 1.3 (p < 0.001)*		4.1 ± 1.4 (p < 0.001)*	
CD4+ T cell count (mean ± SD)	539 ± 304 (Ref)		494 ± 247 (p = 0.55)*		408 ± 179 (p = 0.09)*		260 ± 126 (p = 0.003)*	
	n (%)	**RRR (95% CI)**	**n (%)**	**RRR (95% CI)**	**n (%)**	**RRR (95% CI)**	**n (%)**	**RRR (95% CI)**
CD4+ T cell count < 350	12 (18)	Reference	16 (17)	0.97 (0.31, 3.06)	30 (29)	1.85 (0.59, 5.83)	24 (42)	3.27 (0.94, 11.43)
Use of ART	33 (50)	Reference	53 (59)	1.43 (0.61, 3.37)	64 (62)	1.64 (0.6, 4.46)	29 (51)	1.04 (0.29, 3.69)
Abnormal vaginal flora (Nugent 4-10)‡	25 (38)	Reference	34 (38)	0.95 (0.39, 2.29)	45 (44)	1.21 (0.48, 3.05)	36 (63)	2.63 (0.85, 8.17)
Bacterial vaginosis (Nugent 7-10)‡	18 (27)	Reference	26 (29)	1.09 (0.45, 2.65)	26 (25)	0.92 (0.38, 2.22)	24 (42)	2.10 (0.66, 6.74)
Yeast vaginitis‡	7 (11)	Reference	20 (22)	2.01 (0.51, 7.94)	32 (31)	3.08 (0.83, 11.37)	7 (12)	0.67 (0.14, 3.16)
Trichomoniasis‡	2 (3)	Reference	1 (1)	0.27 (0.06, 1.31)	11 (11)	2.78 (0.54, 14.24)	4 (7)	1.63 (0.26, 10.01)
Cervicitis (>30 neutrophils/hpf)‡	27 (41)	Reference	31 (34)	0.62 (0.27, 1.42)	41 (40)	0.77 (0.31, 1.94)	23 (40)	0.54 (0.20, 1.44)
CMV genital shedding‡	0 (0)	Reference	3 (3)	0.90 (0.34, 2.44)	6 (6)	0.91 (0.41, 2.02)	1 (2)	0.26 (0.03, 2.31)
HSV-1/2 genital shedding‡	1 (2)	Reference	1 (1)	0.96 (0.05, 18.87)	3 (3)	2.63 (0.27, 26.14)	2 (4)	4.71 (0.35, 62.83)
All genital co-infection combined‡	46 (70)	Reference	61 (68)	0.79 (0.32, 1.92)	86 (83)	1.84 (0.69, 4.87)	46 (81)	1.26 (0.36, 4.43)
Kenya								
# of visits	52		63		56		88	
Log_{10} plasma HIV-1 RNA (mean ± SD)	2.9 ± 0.9 (Ref)		3.1 ± 1.0 (p = 0.39)*		3.6 ± 1.2 (p = 0.003)*		4.3 ± 1.2 (p < 0.001)*	
CD4+ count (mean ± SD)	700 ± 274 (Ref)		598 ± 202 (p = 0.04)*		533 ± 167 (p = 0.002)*		429 ± 156 (p < 0.001)*	

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	n (%)	RRR (95% CI)	n (%)	RR (95% CI)	n (%)	RR (95% CI)	n (%)	RR (95% CI)
CD4+ count < 350	1 (2)	Reference	5 (8)	4.4 (0.44, 44.1)	7 (13)	7.29 (0.80, 66.14)	**27 (31)**	**22.6 (2.72, 187.23)**
Use of ART	3 (6)	Reference	5 (8)	1.33 (0.31, 6.11)	4 (7)	1.60 (0.32, 7.95)	1 (1)	0.19 (0.02, 1.90)
Abnormal vaginal flora (Nugent 4-10)‡	13 (25)	Reference	28 (44)	2.33 (0.84, 6.50)	28 (50)	2.79 (0.98, 7.96)	45 (51)	2.37 (0.79, 7.14)
Bacterial vaginosis (Nugent 7-10)‡	6 (12)	Reference	12 (19)	1.74 (0.33, 9.05)	11 (20)	1.66 (0.30, 9.16	27 (31)	2.40 (0.45, 12.95)
Yeast vaginitis‡	4 (8)	Reference	7 (11)	1.57 (0.42, 5.80)	**13 (23)**	**4.66 (1.52, 14.32)**	6 (7)	1.26 (0.33, 4.86)
Cervicitis (>30 neutrophils/hpf)‡	39 (75)	Reference	42 (67)	0.78 (0.44, 1.36)	31 (55)	0.60 (0.30, 1.21)	55 (63)	**0.44 (0.25, 0.76)**
CMV genital shedding‡	6 (12)	Reference	2 (3)	0.32 (0.05, 2.30)	5 (9)	0.70 (0.13, 3.68)	10 (11)	1.20 (0.27, 5.29)
HSV-2 genital shedding‡	1 (2)	Reference	1 (2)	1.04 (0.16, 6.98)	2 (4)	0.69 (0.11, 4.49)	8 (9)	1.91 (0.37, 9.90)
Any genital co-infection detected‡	33 (63)	Reference	44 (70)	1.05 (0.30, 3.71)	32 (57)	0.82 (0.23, 2.85)	56 (64)	0.97 (0.23, 4.11)

Bold = significant result

* p value for a multinomial regression analysis, compared to undetectable.

‡ Multinomial regression analysis controlled for HIV-1 plasma RNA concentration

Table 2

Univariate associations between genital infections and cytokines using linear regression with generalized estimating equations, stratified by nationality. Results are reported as the $\log_{10}$ difference (95% CI) in cytokine concentrations between women with and without the infection.

	US (n = 56 women at 316 visits)				
	N (%)	**IL-1β**	**IL-6**	**IL-8**	**SLPI**
Abnormal Nugent score	140 (45%)	**0.59 (0.42, 0.77)**	0.06 (-0.11, 0.23)	-0.004 (-0.19, 0.18)	**-0.32 (-0.49, -0.15)**
BV *	94 (30%)	**0.61 (0.41, 0.81)**	0.10 (-0.10,0.30)	-0.05 (-0.25, 0.15)	**-0.32 (-0.50,-0.13)**
Yeast *	66 (21%)	-0.02 (-0.31, 0.27)	-0.16 (-0.37, 0.05)	**0.31 (0.08, 0.54)**	0.15 (-0.08, 0.38)
Trichomoniasis	28 (7%)	**0.65 (0.36, 0.94)**	0.24 (-0.06, 0.55)	**0.27 (0.001, 0.54)**	**-0.31 (-0.60, -0.03)**
Cervicitis *	166 (45%)	**0.27 (0.11, 0.43)**	0.11 (-0.03,0.25)	**0.26 (0.09, 0.43)**	-0.03 (-0.19, 0.13)
CMV *	10 (4%)	0.19 (-0.06, 0.44)	0.18 (-0.1, 0.46)	0.25 (-0.19, 0.68)	0.22 (-0.01, 0.45)
HSV *	7 (3%)	**0.50 (0.15, 0.84)**	0.43 (-0.23,1.09)	**0.53 (0.15, 0.92)**	-0.25 (-0.61, 0.11)
Any genital co-infection *	239 (76%)	**0.54 (0.39, 0.68)**	0.06 (-0.09, 0.21)	**0.23 (0.07, 0.38)**	**-0.21 (-0.40, -0.02)**

	Kenya (n = 80 women at 259 visits)				
	N	**IL-1β**	**IL-6**	**IL-8**	**SLPI**
Abnormal Nugent score	114 (45%)	**0.55 (0.35, 0.75)**	0.19 (-0.02, 0.42)	**0.32 (0.14, 0.51)**	-0.13 (-0.28, .01)
BV *	56 (22%)	**0.59 (0.34, 0.83)**	0.20 (-0.09, 0.49)	**0.30 (0.03, 0.57)**	-0.14 (-0.30,0.02)
Yeast *	30 (12%)	0.16 (-0.18, 0.51)	0.11 (-0.24, 0.47)	0.22 (-0.07, 0.52)	-0.06 (-0.29, 0.16)
Cervicitis *	219 (65%)	0.07 (-0.13, 0.27)	-0.04 (-0.28, 0.20)	0.01 (-0.12,0.16)	-0.07 (-0.20, 0.05)
CMV *	23 (16%)	0.24 (-0.06, 0.53)	**0.26 (0.05, 0.47)**	**0.34 (0.03, 0.64)**	0.07 (-0.21, 0.36)
HSV *	12 (8%)	-0.01 (-0.44, 0.42)	0.22 (-0.11, 0.55)	0.12 (-0.26, 0.50)	-0.17 (-0.40,0.05)
Any genital co-infection *‡	165 (85%)	**0.46 (0.21, 0.72)**	- 0.18 (-0.71, 0.35)	0.14 (-0.08, 0.36)	- 0.17 (-0.41, 0.06)

Bold = significant result

*Different prevalence between US and Kenyan women, $p < .05$

‡Missing complete data from 66 visits, thus denominator is 193 visits

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