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Authors

Huchko, Megan Justine
Woo, Victoria
Liegler, Teri
et al.

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Is there an association between HIV-1 genital shedding and cervical intraepithelial neoplasia 2/3 among women on antiretroviral therapy?

Megan J. Huchko, MD, MPH, **Victoria Woo, MD**, **Teri Liegler, PhD**, **Anna Leddy, BA**, **Karen Smith-McCune, MD, PhD**, **George F. Sawaya, MD**, **Elizabeth A. Bukusi, M.Med, PhD**, and **Craig R. Cohen, MD, MPH**
University of California, San Francisco and Kenya Medical Research Institute, Nairobi, Kenya

Abstract

Objective—Given the high prevalence of cervical intraepithelial neoplasia (CIN) grade 2/3 among HIV-infected women, we sought to examine the relationship between CIN 2/3 and HIV-1 genital shedding among women on highly active antiretroviral therapy (HAART).

Materials and Methods—Paired plasma and cervical wick specimens for HIV-1 RNA measurements were obtained from 44 HIV-infected women (cases) with biopsy-confirmed CIN2/3 and 44 age-matched HIV-infected women with normal cervical findings on colposcopy (controls). All subjects tested negative for sexually transmitted infections and had been stable on HAART for at least three months. HIV-1 viral load was measured in both blood and cervical specimens using commercial real-time PCR assays.

Results—CIN2/3 was not significantly associated with the detection or magnitude of plasma or cervical HIV-1 RNA shedding. HIV was detected in the plasma in 10 (23%) cases and 10 (25%) controls (OR=1.0 95% CI 0.33–3.1). Cervical HIV-1 was detected in 6 (13.6%) cases and 9 (20.4%) controls (OR= 0.61 95% CI=0.20–1.90). Mean HIV-1 concentration in cervical secretions among women with CIN2/3 who shed was 2.93 $\log_{10}$ copies versus 2.72 among controls (p=0.65).

Conclusions—Among women on HAART, we found no relationship between CIN 2/3 and HIV-1 genital shedding.

Keywords

HIV-1 Genital Shedding; Highly Active Anti-Retroviral Therapy; Cervical Intraepithelial Neoplasia 2/3

Introduction

The presence and amount of human immunodeficiency virus (HIV) in the plasma and genital tract play a key role in intrapartum and sexual HIV transmission.[1, 2] Identification of modifiable factors associated with genital HIV shedding is critical to the development of effective intervention strategies that target the elimination of the viral reservoir in the genital tract and hence the prevention of HIV transmission. Detectable plasma HIV, sexually transmitted infections, inflammation, cervical ectopy, and genital ulceration have all been consistently shown to be associated with increased concentration of HIV-1 in the female

Corresponding Author: Megan J. Huchko, University of California, San Francisco, Department of Obstetrics and Gynecology, 50 Beale St, Ste 1200, San Francisco, CA 94105, Megan.huchko@ucsf.edu, Phone: 415-597-9318, FAX: 415-597-9200.

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genital tract.[3–9] Conversely, adherence to effective highly active antiretroviral therapy (HAART) has been shown to dramatically reduce the concentration of HIV in the genital tract.[10–12]

HIV-infected women are at increased risk for infection with human papillomavirus (HPV) and development of cervical intraepithelial neoplasia (CIN). [13] The rate of CIN has been estimated to be two to three times as great among HIV-infected women compared to HIV-negative women.[14, 15] Despite the high prevalence of CIN and cancer in this population, remarkably little is known about the relationship between HPV-related lesions, treatment of these lesions and HIV-1 genital shedding. One prospective study of women not on HAART showed an up to 4-fold risk of HIV-1 RNA in the genital tract among women with oncogenic HPV types, with no significant relationship to CIN. However, almost 50% of women in the study had concomitant genital tract infections. [16] A later study by the same investigators showed a two-fold increased risk of HIV-1 shedding in the genital tract among women with CIN, after controlling for plasma concentrations of HIV-1, CD4+ count and vaginal infections.[17] Given the absence or low numbers of women on HAART in these studies, the relationship between CIN2/3 and HIV-1 genital shedding among women on HAART remains unclear. We hypothesized that the protective effect of HAART would diminish or eliminate any association between CIN2/3 and HIV-1 genital shedding. We sought to examine the association between CIN2/3 and HIV-1 genital shedding in women on HAART with no clinical or immunologic evidence of treatment failure.

Materials and Methods

This case-control study was conducted between October 2010 and April 2011 among HIV-1 seropositive women attending the Family AIDS Care and Education Services (FACES) clinic in Kisumu, Kenya. Ethical approval was obtained from the University of California, San Francisco (UCSF) Committee on Human Research and the Kenya Medical Research Institute (KEMRI) Ethical Review Committee. Women interested in cervical cancer screening were recruited for this study if they were over 23 years of age (FACES criteria for cervical cancer screening), not pregnant, reported no intercourse in the three days prior to their visit and had been on the same HAART regimen for at least three months with >90% self-reported adherence and no clinical or immunologic evidence of treatment failure.[18] All participants were on the standard HAART regimens available at FACES, per the Kenyan Ministry of Health Guidelines. First-line nucleoside reverse transcriptase (NRTI) based regimens contained zidovudine or stavudine plus lamivudine plus a non-nucleoside reverse transcriptase inhibitor (NNRTI), either nevirapine or efavirenz. Second-line NRTI-based regimens included tenofovir, plus lamivudine and an NNRTI. Third-line protease inhibitor (PI) based regimens contained lopinavir/ritonavir in combination with two other antiretrovirals.

Potential participants signed an informed consent prior to undergoing pelvic examination with specimen collection for measurement of cervical HIV-1 RNA, high-risk HPV and sexually transmitted infections (STIs), followed by visual inspection with acetic acid (VIA) and colposcopy. Colposcopy was done on all participants regardless of VIA results. Two study clinicians who had undergone didactic and practical training, and had each performed over 200 exams prior to study initiation performed all VIA and colposcopic examinations. Women with negative colposcopy (satisfactory colposcopy with no aceto-white lesions seen and no biopsy performed) were determined to have no CIN2/3 (controls); CIN2/3 diagnosis was made on the basis of colposcopically directed biopsy result (cases). Baseline clinical and demographic variables were collected for each woman at time of procedure, including age, CD4+ cell count, World Health Organization (WHO) clinical stage, anti-retroviral therapy regime and duration, hormonal contraception use, menstrual phase, gravidity, parity

and number of current and lifetime number of sexual partners. Women then had blood collected for plasma HIV-1 viral load. Final study eligibility was based on absence of clinically evident cervicitis, vaginitis or genital ulcers and absence of laboratory confirmed *Trichomonas vaginalis, Chlamydia trachomatis or Neisseria gonorrhoeae.* Cases were sequentially identified among eligible women who had biopsy-confirmed CIN2/3. Controls were then chosen among eligible women who had a colposcopically normal cervical examination (i.e. satisfactory colposcopy examination with no aceto-white lesions or visual impression of CIN1) performed during the same time period and matched to the cases' age ± two years.

Specimen collection and HIV-1 RNA viral load testing on blood plasma and cervical secretion

Blood plasma and cervical secretions were collected from eligible participants at study enrollment for HIV-1 RNA viral load testing. Processed blood plasma was stored at −80°C for up to 3 months in the FACES laboratory before testing at the KEMRI Pathology and Oncology Research Laboratory in Nairobi using the Abbott RealTi*m*e Viral Load assay (Abbott Molecular, Des Plaines, IL) with a lower limit of detection (LLOD) of 40 copies per mL. Cervical fluid samples were collected immediately after speculum insertion by inserting three Tear Flo strips (Odyssey Medical, Bartlett, TN) into the cervical os until approximately 20 mm of fluid was absorbed. Strips were withdrawn and cut at the 15 mm line, collecting approximately 12 μL fluid per wick or 36 μL per participant. Wicks were immediately immersed in 1.0 mL of Abbott lysis buffer solution and stored at −80°C for up to four months before being shipped on dry ice for testing at the AIDS Research Institute-UCSF Laboratory of Clinical Virology using the Abbott RealTi*m*e Viral Load assay. Cervical wick VL results are presented as $\log_{10}$ number of copies per specimen (cps) consisting of 3 wicks per participant.

Measurement of high risk HPV and STIs

After collecting wick specimens for cervical HIV-1, clinicians inserted a cytobrush into the cervical os, rotated it twice, swabbed it over the ectocervix and then placed it into a cryovial with normal saline for high-risk HPV testing. Next, a single Dacron swab was placed in the cervical os and gently rotated for 10–30 seconds, removed, and placed in the transport media for STI testing. HPV specimens were then shipped on dry ice to the German Cancer Research Center for detection of high-risk HPV types using Hybrid Capture 2™ (Qiagen, Shanghai, China). STI specimens were stored at room temperature in the FACES laboratory until shipment to Nairobi for analysis for *T. vaginalis* (InPouch,™ Biomed Diagnostics, White City, Oregon)*, C. trachomatis and N. gonorrhoeae* (Cobas® Amplicor, Roche, Inc, West Sussex, England).

Statistical analysis

The primary outcome for this analysis was presence or absence of HIV-1 RNA in the cervical fluid specimens. Assumptions for sample size calculations were based on previous studies of variation in HIV-1 RNA levels within women,[19] prevalence of HIV-1 genital shedding among women on HAART [10, 20] and the earlier studies showing a difference in detectable shedding of 24–26% between women with high-risk HPV or any CIN compared to no HPV infection and normal cervical cytology, respectively.[16, 17] Extrapolating from the results of these studies, we anticipated a 25% difference in frequency of detecting HIV-1 in cervical secretions among women on HAART with CIN2/3 in comparison to normal colposcopy, and considered a difference of that magnitude to be clinically significant. We calculated a sample size of 42 matched-pairs to provide 80% power with a two-sided alpha of 0.05 to detect that difference.

Clinical and demographic variables were collected at the time of the study visit, through participant interview and electronic and paper chart review. Study variables were double entered into a database and spreadsheet (MS Access and Excel, MicroSoft Inc., Redmond, WA). Univariate analyses using Student's t-test, Mann-Whitney U-tests and Pearson's chi-square were performed to compare clinical and demographic characteristics between the cases and controls. For multivariable analysis we used logistic and linear regression to explore potential factors associated with presence and concentration of HIV-1 in cervical secretions. Variables determined to be significantly different between cases and controls were controlled for in the final regression models. All statistical analysis was performed with STATA 11 (StataCorp, College Station, TX, USA).

Results

During the recruitment and enrollment period, 54 (14%) women were identified as eligible cases and 155 (40%) women as potential controls, from 385 women who consented to undergo screening (Figure 1). The main reasons for ineligibility were vaginal infection and visual impression of CIN1 at the time of colposcopy. Among the 209 women eligible as cases and controls, 46 age-matched pairs were identified, and had plasma and cervical specimens analyzed for HIV-1 viral load. Cervical viral load results were obtained from 88/92 (96%) specimens, four specimens had indeterminate results due to PCR inhibition as indicated by internal control failure. Results are presented from the 44 matched pairs.

Overall, mean age for participants was 32.8 years (SD 6.64); median CD4+ T-cell count 367 cells/mL (range 7–1366 cells/mL) and median time on HAART was 11 months (range 3–100 months). Cases had significantly lower CD4+ counts, were more recently diagnosed with HIV, had been on their current HAART regimen for less time and were more likely to have oncogenic HPV detected compared to controls. Overall two thirds of participants were on a first-line nucleoside reverse transcriptase (NRTI) based regimen. Only three participants were on a PI-based regimen, and all three were cases (Table 1).

The diagnosis of CIN2/3 was not associated with HIV-1 RNA detection or concentration in cervical secretions. Cervical HIV-1 was detected in 6 (13.6%) cases and 9 (20.4%) controls (OR= 0.61 95% CI=0.20–1.90). (Table 2) Among the women with detectable HIV-1 cervical shedding, the mean concentration was 2.93 $\log_{10}$cps in cases versus 2.72 $\log_{10}$cps among controls (p=0.65). After adjusting for serum HIV viral load, CD4+ count, time since HIV diagnosis, duration of current HAART regimen and HPV positivity, CIN2/3 was not associated with detection (Adjusted (A)OR=0.52, 95% CI=0.12–2.24) or concentration (0.44 log10cps, 95% CI=−16.89–17.76) of detectable HIV-1 in cervical shedding. (Table 3)

Detectable plasma HIV-1-RNA was present in 10 (23%) cases and 10 (23%) controls (OR=1.0 95% CI 0.33–3.1). There was no difference in the plasma HIV-1 RNA concentration between cases and controls (3.14 log cps vs. 2.65 log cps, p=0.42). Seven of the 15 (47%) participants with detectable cervical HIV-1 RNA had undetectable plasma HIV-1 RNA. After stratifying by case status, detectable plasma HIV-1 RNA was only statistically associated with HIV-1 cervical shedding among controls (OR 7.5, 95% CI=1.5–38.0), but not cases (OR 3.75, 95% CI 0.63–22.2). No other clinical or demographic variables were significantly associated with detection of HIV-1 RNA in cervical secretions.

Conclusions

We did not find an association between CIN2/3 and detection or magnitude of HIV-1 cervical shedding in women who were on HAART with no clinical or immunologic evidence of treatment failure. This finding is in contrast to previous studies looking at

associations between either CIN or HPV and HIV in genital secretions.[16, 17] One explanation for this difference may be due to the low proportions of women on HAART in the earlier works. In a previous study, in which there were 46 cases of CIN2/3 and 155 cases of any CIN, only 37.5% of women were on HAART. Although the authors found an association between HIV-1 shedding and any CIN diagnosis, HAART was not seen to impact this relationship. [17] Using CIN2/3 as an outcome, we anticipated a significant association with HIV-shedding given the increased severity of the lesion. The absence of an association in this adequately powered study suggests that effectiveness of and adherence to HAART attenuates the relationship between CIN2/3 and HIV-1 shedding.

Our study population demonstrated similar or lower levels of detectable plasma and cervical shedding than seen in other studies among women on HAART.[21, 22] Although our numbers were too small to make a statistically significant correlation, half of women with undetectable plasma HIV-1 RNA had detectable HIV-1 RNA in cervical secretions despite the absence of sexually transmitted infections. This supports the hypothesis that although plasma viral load is an important driver of detectable genital viral load, the genital tract is a biologically distinct compartment, in which there may be distinct risk factors for HIV presence or replication.[23]

The cross-sectional case-control format limits the generalizability and direction of association in this study. It is possible that the factors leading to increased risk of CIN2/3 also would have contributed to increased levels of HIV-1 in the cervicovaginal secretions (Type I error). The cases in our study had significantly lower CD4+ counts, had been more recently diagnosed with HIV, had been on their current HAART regimen for less time and were more likely to be high-risk HPV positive, all of which would are known risk factors for CIN2/3 among HIV-infected women. To minimize the contribution of these clinical differences in the association between CIN2/3 and HIV-1 cervical shedding, we matched participants on age and controlled for potential confounding variables in our regression models. It is possible that unmeasured confounding remained or that we had limited power to definitively identify some associations that may exist.

One of the strengths of this study that differs from previous work was sensitive detection of HIV-1 viral load to 40 cps.[24] In contrast to previous studies using up to 1000 cps as the lower limit of detection for both genital and plasma secretions, our cut-off of 40 cps allowed us to have more precision in our measures of association.[16, 17] We also eliminated potential confounding by enrolling women on HAART and without clinical or laboratory evidence of sexually transmitted infections. Although this may limit the generalizability of the findings to other populations, we chose this design to specifically investigate the relationship between CIN2/3 and HIV-1 shedding among women on HAART.

A better understanding of the impact of CIN on HIV-1 genital shedding may provide additional rationale for implementing cervical cancer screening programs in resource-limited settings, where populations are at high-risk for both cervical cancer and HIV transmission. Because this was a case-control study, we would not make recommendations for change in clinical practice based on our findings. However, given the high co-prevalence of CIN2/3 and HIV in certain areas of the world, these findings provide some reassurance that affected women are not likely to be at increased risk of HIV transmission. This study provides preliminary data for prospective studies looking at risk factors for transmission, risk of HIV-1 shedding among women undergoing diagnosis and treatment for CIN2/3, and cost-effectiveness and impact analyses of HAART initiation in various populations.

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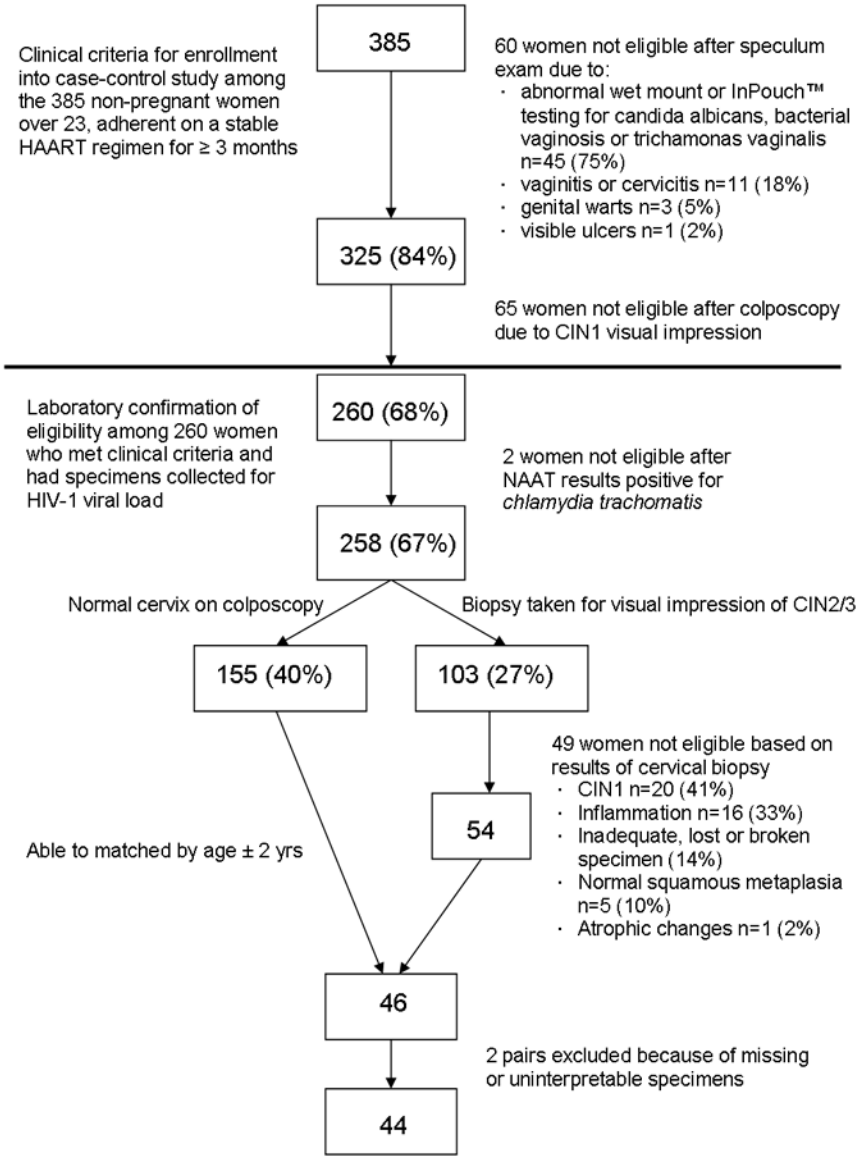

Figure 1.
Eligibility determination for the 44 age-matched pairs

Table 1

Demographic, reproductive and HIV-related characteristics among women diagnosed with CIN2/3 (cases) compared to women without CIN2/3 (controls)

Characteristic	Case (n=44)	Control (n=44)	p-value
Age, mean yrs (SD)	32.3 (6.6)	33.2 (6.7)	0.56
Marital status (%)			
Married	9%	14%	0.08
Single	45%	66%	
Widowed	32%	11%	
Separated	14%	9%	
Parity, mean (SD)	2.5 (.22)	2.9 (.29)	0.22
Using hormonal contraception (%)	27%	23%	0.24
Menstrual cycle phase (%)			
Follicular (day 1–14)	48%	34%	0.41
Luteal (day 15–30)	27%	32%	
Amenorrhea	25%	34%	
Lifetime number of partners, mean (SD)	4.4 (2.9)	4.6 (2.0)	0.73
Previous sexually transmitted infection (%)	20%	18%	0.79
Current symptoms of vaginal discharge, odor or pruritus * (%)	20%	25%	0.61
CD4+ count, mean cells/mL (SD)	333 (274)	461 (273)	0.03
WHO Stage (%)			
1	18%	16%	0.91
2	24%	27%	
3	39%	43%	
4	18%	14%	
Time since HIV Diagnosis, mo. (SD)	29.7 (20)	42.6 (29.6)	0.02
Time on current HAART regimen, mo. (SD)	16.0 (18.4)	25.4 (21.4)	0.03
Previous HAART regimen (%)	34%	30%	0.64
HAART Regimen †			
1st line NRTI	68%	66%	0.16
2nd line NRTI	25%	34%	
3rd line PI	7%	0%	
Detectable plasma viral load (%)	25%	23%	0.80
Median plasma viral load, log copies/ml (IQR), n=20	1.9 (1.8–3.2)	3.3 (1.9–3.7)	0.25
High-risk HPV positive (%), n=68	86%	42%	0.001

* Women with evidence of cervicitis, abnormal vaginal discharge, abnormal results on wet mount or laboratory confirmed *Neisseria gonorrhoeae*, *Chlamydia trachomatis* or *Trichomonas vaginalis* were excluded from the study

† 1st line Non-nucleoside Reverse Transcriptase Inhibitors: AZT/D4T + 3TC + NVP/EFV, 2nd line NRTI: TDF + 3TC + NVP/EFV, 3rd line PI: LPV/RTV + 3TC + NRTI

Table 2

Cervical and plasma HIV-1 viral load levels* among individual women with CIN2/3 (cases) compared to those without CIN2/3 (controls)

	Cases			Controls		
	Case ID#	**Cervical**	**Plasma**	**Control ID#**	**Cervical**	**Plasma**
Detectable HIV-1 in plasma samples only	2	ND	1.96	1	ND	1.88
	3	ND	1.86	5	ND	1.85
	5	ND	2.56	21	ND	5.34
	39	ND	1.88	26	ND	1.66
	24	ND	1.79	18	ND	2.00
	28	ND	3.19			
	57	ND	1.76			
Detectable HIV-1 in cervical samples only	13	1.93	ND	4	2.88	ND
	17	2.27	ND	10	4.80	ND
	27	3.12	ND	33	1.61	ND
				40	2.85	ND
Detectable HIV-1 in both cervical and plasma samples	23	3.10	4.91	12	3.25	3.20
	32	3.91	5.74	15	2.36	3.56
	33	2.00	1.83	17	2.31	4.76
				31	3.38	3.46
				39	2.95	3.75

*Concentration $\log_{10}$ HIV-1 RNA

ND=not detectable HIV-1 RNA (<40 copies/mL in plasma, <40 cps in cervical secretions)

Table 3

Association between detection and concentration of cervical HIV-1 RNA and CIN2/3

	Unadjusted Odds Ratio	Adjusted Odds Ratio†
Odds of cervical HIV-1 RNA detection among women with CIN2/3	0.60 (95% CI=0.16–2.12)	0.63 (95% CI=0.11–3.83) *
	Unadjusted (log_{10}cps)	Adjusted (log_{10}cps) †
Relative change in cervical HIV-1 RNA concentration among women with CIN2/3	−0.21 (95% CI=−1.17–0.75)	0.43 (95% CI= −16.89–17.76) *

* Not-significant

† Both models adjusted for serum viral load, CD4+ cell count, time since HIV diagnosis, duration of current HAART regimen and high-risk HPV positivity