Increased Cervical Human Immunodeficiency Virus (HIV) RNA Shedding Among HIV-Infected Women Randomized to Loop Electrosurgical Excision Procedure Compared to Cryotherapy for Cervical Intraepithelial Neoplasia 2/3

Sharon A. Greene,[1,2] Christine J. McGrath,[1] Dara A. Lehman,[1,3] Kara G. Marson,[1] T. Tony Trinh,[1] Nelly Yatich,[1] Evans Nyongesa-Malava,[1] Catherine Kiptinness,[1] Barbra A. Richardson,[1,4,5] Grace C. John-Stewart,[1,2,6] Hugo De Vuyst,[7] Samah R. Sakr,[8] Nelly R. Mugo,[1] and Michael H. Chung[1,2,6]

[1]Department of Global Health and [2]Department of Epidemiology, University of Washington, [3]Division of Human Biology, Fred Hutchinson Cancer Research Center, [4]Department of Biostatistics, University of Washington, [5]Division of Vaccine and Infectious Diseases, Fred Hutchinson Cancer Research Center, and [6]Department of Medicine, Division of Allergy and Infectious Diseases, University of Washington, Seattle; [7]Infection and Cancer Epidemiology Group, International Agency for Research on Cancer, Lyon, France; and [8]Coptic Hospital, Nairobi, Kenya

Background. Treatment of human immunodeficiency virus (HIV)–infected women to prevent cervical cancer may stimulate HIV RNA cervical shedding and risk HIV transmission.

Methods. From 2011 to 2014, 400 HIV-infected women diagnosed with cervical intraepithelial neoplasia 2/3 in Kenya were randomized to loop electrosurgical excision procedure (LEEP) or cryotherapy. Cervical samples were collected at baseline and 3 weekly intervals. Samples were tested for HIV RNA using the Gen-Probe Aptima HIV assay with a minimum detection level of 60 copies/swab and analyzed using generalized estimating equations.

Results. Women who received LEEP had significantly higher cervical HIV RNA levels than those who received cryotherapy at weeks 2 (adjusted incident rate ratio [aIRR], 1.07; $P = .038$) and 3 (aIRR, 1.08; $P = .046$). Within LEEP, significantly higher cervical shedding was found at weeks 2 (2.03 $\log_{10}$ copies/swab; $P < .001$) and 3 (2.04 $\log_{10}$ copies/swab; $P < .001$) compared to baseline (1.80 $\log_{10}$ copies/swab). Cervical HIV RNA was significantly higher following LEEP for up to 3 weeks among women on antiretroviral treatment (ART) (0.18 $\log_{10}$ copies/swab increase; $P = .003$) and in ART-naive women (1.13 $\log_{10}$ copies/swab increase; $P < .001$) compared to baseline. Within cryotherapy, cervical shedding increased in ART-naive women (0.72 $\log_{10}$ copies/swab increase; $P = 0.004$) but did not increase in women on ART.

Conclusions. Women randomized to LEEP had a larger increase in post-procedural cervical HIV shedding than cryotherapy. Benefits of cervical cancer prevention outweigh the risk of HIV sexual transmission; our findings underscore the importance of risk-reduction counseling.

Clinical Trials Registration. NCT01298596.

Keywords. cervical cancer; cervical HIV shedding; HIV; loop electrosurgical excision procedure (LEEP); cryotherapy.

Women infected with human immunodeficiency virus (HIV) experience a higher burden of cervical cancer than HIV-uninfected women [1, 2]. Cervical cancer prevention depends on early diagnosis, treatment, and continued clinical follow-up. Globally, standard treatment modalities for cervical intraepithelial neoplasia (CIN) include loop electrosurgical excision procedure (LEEP) and cryotherapy. LEEP is the standard of care for treatment of CIN in the United States and Europe [3, 4], and cryotherapy is commonly used in resource-limited settings that use a single-visit "see-and-treat" approach [5–10].

Treatment of CIN can cause inflammation or ulceration of the cervix [11, 12]. Increased risk of hemorrhage associated with LEEP and watery discharge with cryotherapy have been reported [13]. In HIV-infected women, these interventions may stimulate cervical shedding of HIV and subsequently increase the risk of HIV sexual transmission from treated women to HIV-uninfected partners [14]. While the risk of sexual HIV transmission from women on antiretroviral therapy (ART) is low, cervical HIV levels can differ from plasma HIV levels [15–20]. Both LEEP and cryotherapy have been associated with cervical shedding of HIV [11, 21–23]. However, they have never been directly compared to each other, and few studies have rigorously examined cervical shedding after these procedures in association with ART use, plasma viral load, and immunodeficiency [24].

We conducted a randomized, controlled trial (RCT) to compare recurrence of cervical disease among women with high-grade

Received 5 September 2017; editorial decision 3 December 2017; accepted 17 December 2017; published online December 20, 2017.

Correspondence: S. A. Greene, Department of Global Health, University of Washington, NJB 13th Floor, 325 9th Avenue Box 359909, Seattle, WA 98104 (sharonag@uw.edu).

Clinical Infectious Diseases® 2018;66(11):1778–84

DOI: 10.1093/cid/cix1096

precancerous cervical lesions randomized to LEEP or cryotherapy [25]. Within the parent trial, we nested a substudy to compare the cervical HIV RNA levels at 1, 2, and 3 weeks following LEEP or cryotherapy and hypothesized that LEEP would be more likely to increase cervical HIV RNA levels compared to cryotherapy.

METHODS

Study Population

This study was nested within an RCT to compare recurrence of cervical lesions between LEEP and cryotherapy over 2-year follow-up. The RCT was performed at the Coptic Hope Center for Infectious Diseases, an urban HIV clinic in Nairobi, Kenya, that offers free, comprehensive HIV care and treatment with support from the President's Emergency Plan for AIDS Relief [26]. Eligible HIV-infected women enrolled at the Coptic Hope Center who agreed to participate in the study were consented and screened for cervical cancer using Papanicolau (Pap) smear and colposcopy-directed biopsy. Women were eligible for the study if they were HIV infected, aged ≥18 years, had initiated sexual activity, were not pregnant, and had an intact cervix without history of hysterectomy. Women were excluded if they had a history of treatment for cervical lesions or if they had a lesion that was larger than 75% of the cervix surface or extended more than 2 mm into the endocervical canal. Women with a high grade squamous intraepithelial lesion or squamous cell carcinoma by Pap smear and histologically confirmed CIN2/3 disease were randomized.

Randomization and Follow-up

Randomization was prepared using a computer-generated sequence in permuted blocks of 10, and assignment was in a 1:1 allocation to receive LEEP or cryotherapy. Study investigators and participants were not blinded to the interventions. During the baseline visit, women completed a standardized questionnaire that detailed medical, sexual, and reproductive histories. ART use and duration were abstracted from the patient health record at the Coptic Hope Center.

Prior to treatment, endocervical swabs for HIV RNA were obtained by placing a sterile Dacron swab (Puritan, Guilford, Maine) on the outer part of the endocervix and rotating 360 degrees in the cervical os. One swab was kept dry and another swab was expressed into a cryotube that contained 1 mL of freezing media (70% Roswell Park Memorial Institute 1640 medium, 20% fetal bovine serum, and 10% dimethyl sulfoxide; Sigma Life Science, St. Louis, Missouri). A blood sample was also collected for CD4 lymphocyte counts and plasma HIV RNA.

The cervix was colposcopically visualized, and Lugol's solution was applied to outline the lesion and transformation zone. Prior to LEEP, the cervix was injected with 3–5 mL of anesthetic and Lugol's solution. A high-frequency electrosurgical generator, Finesse II (Utah Medical Products Inc., Midvale, Utah) was inserted at a depth of 4–5 mm and drawn laterally across the cervix, and an additional pass with the loop was made only for large lesions to ensure complete excision. The study doctor performed cryotherapy using nitrous oxide with a 3-minute freeze, 5-minute thaw, and 3-minute freeze method.

The duration of treatment allowed for ice crystal formation that extended 4–5 mm beyond the tip of the cryotherapy to ensure tissue destruction of up to 5 mm in depth. Appropriate 25 mm or 19 mm probes with either a flat surface or a shallow nipple were used according to the size and location of the lesion. Hemostasis was achieved following LEEP with electrocoagulation or application of Monsel's paste, or both. Following cryotherapy, Monsel's paste was applied if bleeding was observed. Women were advised to abstain from sexual activity for 4 weeks following treatment. The same study doctor performed all clinical procedures.

Women were scheduled for follow-up in the study clinic at 7, 14, and 21 days after treatment. At each time point, cervical and plasma samples were collected, a physical examination was performed, and a standardized questionnaire was administered on acceptability of treatment and sexual activity.

Laboratory Testing

Cervical and plasma samples were stored at 80°C and shipped to the Fred Hutchinson Cancer Research Center in Seattle, Washington, on dry ice. HIV-1 RNA was quantified in cervical and plasma specimens using Gen-Probe APTIMA HIV assay (Hologic Gen-Probe Inc., San Diego, California). Specimens with <300 μL of cervical fluid were not sufficient for testing. The lower limit of HIV RNA detection was 60 copies/swab for cervical samples and 60 copies/mL for plasma samples. Samples that measured below the lower limit of detection were assigned the median value, 30 (1.48 $\log_{10}$) copies, between zero and the lower limit of detection. CD4 lymphocyte counts were determined using flow cytometry (Becton Dickinson Biosciences, San Jose, California).

Statistical Analyses

The targeted sample size of the parent RCT, 400 women (200 per arm), was designed to detect a 10% difference in recurrence of CIN between treatment arms. This sample size was sufficient to detect a 0.25 $\log_{10}$ difference in cervical HIV RNA levels with >90% power. Cervical HIV shedding was compared between arms using intent-to-treat analysis.

Statistical analyses were performed using Stata version 14.1 (Stata Corp., College Station, Texas). Generalized estimating equations with robust standard errors were used to model the mean change in cervical HIV RNA levels over time. We assumed an exchangeable correlation structure to account for intrasubject correlation and used a log link to interpret relative change and an identity link for absolute change in risk.

Cervical HIV RNA was modeled as a log-transformed, continuous outcome. Weekly follow-up study visits were categorical variables defined as any study visit ±3 days of 7-, 14-, or 21-day time points post-treatment. The multivariate model included baseline cervical HIV RNA, an interaction term for treatment and time, detectable plasma HIV RNA, and dichotomous ART (on/off). We conducted subanalyses to evaluate change in cervical HIV RNA levels by treatment arm. Each treatment arm was also stratified by women on or off ART because it was expected HIV RNA levels would vary by ART exposure.

The ethical review committees of the University of Washington and Kenyatta National Hospital approved the protocol.

RESULTS

Baseline Characteristics

Demographic and behavioral characteristics were balanced between the treatment arms (Table 1). Fewer women treated with cryotherapy had baseline CIN3, 54.5% (n = 109) compared to 64.0% (n = 128) among women treated with LEEP. Baseline median CD4 count was 380 (interquartile range, 215–525) cells per mm^3 in the overall cohort and did not differ significantly between the arms. Seventeen women (8.5%) in LEEP and 28 (14%) in cryotherapy were off ART at the time of intervention. Most women (79.8%) reported exposure to ART for longer than 6 months. At baseline, plasma viral load was similar between LEEP and cryotherapy (mean $\log_{10}$ HIV RNA, 2.17 vs 2.33; $P = .224$), and cervical shedding was significantly lower in LEEP (mean $\log_{10}$ HIV RNA, 1.80 vs 2.02; $P = .009$).

Cervical HIV RNA Following LEEP vs Cryotherapy

Among 400 women randomized to LEEP or cryotherapy in the parent RCT, 2 women (1.0%) in the LEEP and 3 (1.5%) in the cryotherapy arm had inadequate specimens at baseline. Of 600 possible scheduled follow-up visits in the LEEP arm, 18 (3.0%) visits were missed, 51 (8.5%) were outside the ±3-day window, and 9 specimens were inadequate (1.5%). In the cryotherapy

Table 1. Baseline Demographic, Behavioral, and Clinical Characteristics by Treatment Arm

	Cryotherapy		Loop Electrosurgical Excision Procedure	
Characteristic	n	Median (IQR) or %	n	Median (IQR) or %
Age (years)	200	38.2 (32.0–42.5)	200	37.4 (31.7–44.9)
Education (years)	200	11.0 (8.0–12.0)	200	11.0 (8.0–12.0)
Marital status	200		200	
Single	58	29.0	52	26.0
Committed relationship	79	39.5	84	42.0
Divorced/Separated/Widowed	63	31.5	64	32.0
Salaried employment	53	26.5	63	31.5
Monthly income	186		193	
<$50	44	23.7	48	24.9
$50–$150	65	35.0	69	35.8
>$150	77	41.4	76	39.4
Lifetime sexual partners	197		198	
1–2	88	44.6	67	33.8
≥3	93	47.2	113	57.0
Any hormonal contraception	36	18.1	32	16.0
Menses in last 7 days	39	20.1	33	16.8
Cervical intraepithelial neoplasia 3	109	54.5	128	64.0
CD4 (cells/mm^3)	200	371 (213–532)	199	385 (221–522)
<250	59	29.5	59	29.7
250–499	83	41.5	84	42.2
≥500	58	29.0	56	28.1
No ART exposure	28	14.0	17	8.5
ART exposure <30 days	3	1.5	6	3.0
ART exposure 30 days–6 months	12	6.0	15	7.5
ART exposure >6 months	157	78.5	162	81.0
Median ART exposure (months)[a]	172	29.4 (14.1–60.2)	183	34.0 (12.4–74.3)
Plasma HIV RNA ≥1.48 $\log_{10}$ copies/mL[c]	72	37.0	67	34.7
Cervical HIV RNA ≥1.48 $\log_{10}$ copies/swab[b]	61	31.0	42	21.2

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range.

[a]Among those on ART prior to treatment.

[b]Minimum limit of detection of HIV RNA was 1.48 $\log_{10}$ copies/mL for plasma and 1.48 $\log_{10}$ copies/swab for cervical swabs.

arm, 17 (2.8%) visits were missed, 51 (8.5%) visits were outside the ±3-day window, and 9 (1.5%) had inadequate specimens of <300 µL of cervical fluid (Figure 1). Fewer women in the LEEP arm engaged in sexual intercourse in the 3 weeks following cervical treatment, 33 (16.6%) compared to 42 (21.1%) in cryotherapy.

There was no difference in mean cervical HIV RNA levels between arms in the first (incident rate ratio [IRR], 0.95; 95% confidence interval [CI], 0.87–1.04; P = .297), second (IRR, 0.98; 95% CI, 0.88–1.09; P = .727), and third weeks (IRR, 0.98; 95% CI, 0.87–1.10; P = .701) after cervical treatment in the unadjusted analysis (Figure 2).

Adjusting for baseline cervical HIV RNA, ART exposure, and plasma viral load, the mean rate of change in cervical shedding from baseline was similar between treatments at week 1 (aIRR, 1.04; 95% CI, 0.98–1.10; P = .203). However, the risk of HIV cervical shedding in the LEEP arm was significantly increased compared to the cryotherapy arm at week 2 (aIRR, 1.07; 95% CI, 1.00–1.15; P = .038) and remained significant at week 3 (aIRR, 1.08; 95% CI, 1.00–1.16; P = .046). The magnitude of difference in mean cervical HIV RNA levels between the treatment arms in the adjusted analysis was consistently higher in the LEEP arm and ranged from 0.03 to 0.12 $\log_{10}$ copies during follow-up.

Cervical HIV Shedding Within the LEEP Arm

Women treated with LEEP showed a significant increase in mean cervical HIV RNA between week 1 and baseline from 1.80 to 1.92 $\log_{10}$ copies (P = .009). Cervical HIV RNA levels further increased at week 2 to 2.03 $\log_{10}$ copies (P < .001) and week 3 to 2.04 $\log_{10}$ copies (P < .001). LEEP's effect on cervical HIV RNA levels remained statistically significant after adjusting for plasma HIV RNA levels in multivariate analysis. Among the subset of women on ART who were treated with LEEP, the mean cervical viral load increased from baseline at weeks 2 and 3 (week 2 mean HIV RNA increased to 1.88 $\log_{10}$ copies [P = .002] and at week 3, 1.93 $\log_{10}$ copies [P = .002]). Among women off ART, cervical HIV RNA levels increased from a baseline of 2.29 $\log_{10}$ copies to 3.02 $\log_{10}$ copies (P < 0.001) at week 1, 3.56 $\log_{10}$ copies (P < 0.001) at week 2, and 3.36 $\log_{10}$ copies (P < 0.001) at week 3 (Table 2).

Cervical HIV Shedding Within the Cryotherapy Arm

Mean cervical HIV RNA levels for women treated with cryotherapy were 2.02 $\log_{10}$ copies at baseline and did not significantly increase at week 1 (1.96 $\log_{10}$ copies; P = .566), week 2 (2.04 $\log_{10}$ copies; P = .150), or week 3 (2.12 $\log_{10}$ copies; P = .122; Table 3). The lack of association persisted in analyses adjusting for plasma viral load. Among the subset of women on ART randomized to cryotherapy, cervical HIV RNA levels did not increase at weeks 1 (1.82 $\log_{10}$ copies; P = .627), 2 (1.86 $\log_{10}$ copies; P = .877) 2, or 3 (1.86 $\log_{10}$ copies; P = .831) above baseline. Among women off ART, mean cervical HIV RNA was unchanged at week 1 (2.83 $\log_{10}$ copies; P = .812) following cryotherapy. However, at weeks 2 and 3, mean HIV RNA levels

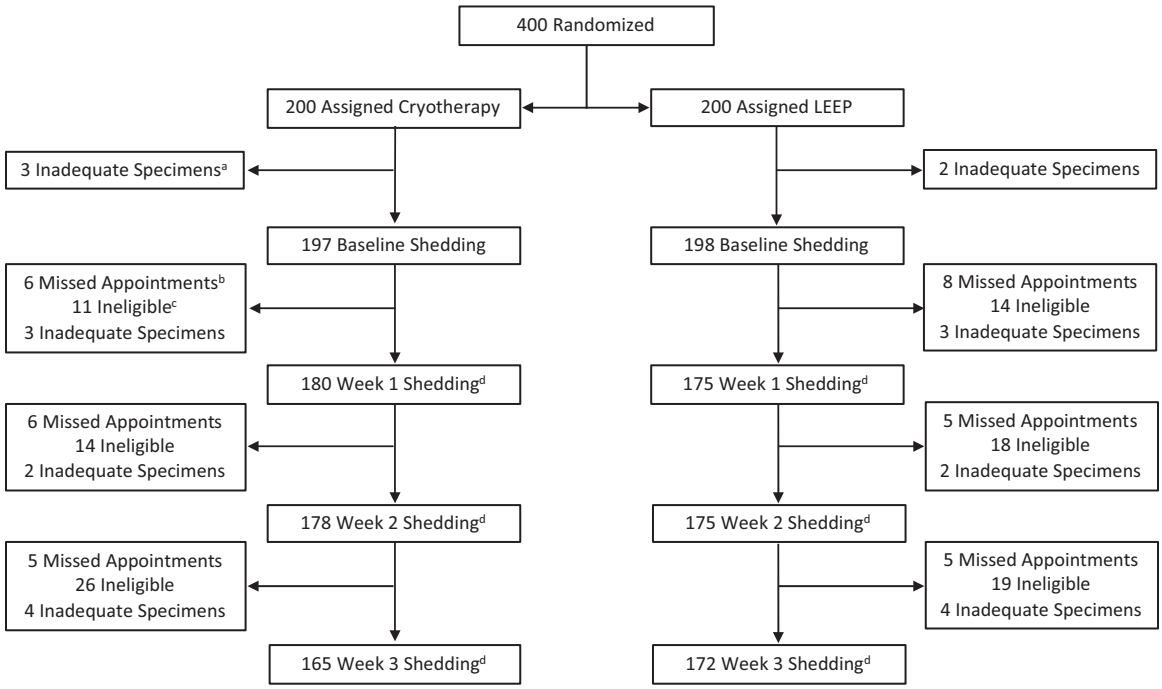

Figure 1. Participant flow of randomization and follow-up in the trial. [a]The cervical swab volume collected was <300 µL. [b]The study participant did not attend a follow-up study visit at week 1, week 2, or week 3. [c]Follow-up study visits did not occur within ±3 days of 7, 14, or 21 days post-treatment. [d]The expected number of women at weeks 1, 2, and 3 was 200 for each arm. Abbreviation: LEEP, loop electrosurgical excision procedure.

Table 2. Change in Mean Cervical Human Immunodeficiency Virus RNA (log_{10} copies/swab) Among Women Treated With Loop Electrosurgical Excision Procedure

Time	Mean (copies/ swab)	Absolute Mean Change From Baseline (95% CI)[a]	Unadjusted IRR[b] (95% CI)	*P* Value	Adjusted IRR[b] (95% CI)	*P* Value
Overall[c] (n = 200)						
Baseline	1.80	-	-		-	-
Week 1	1.92	0.10 (0.02–0.18)	1.06 (1.01–1.10)	.009	1.06 (1.01–1.11)	.010
Week 2	2.03	0.25 (0.14–0.37)	1.14 (1.08–1.21)	<.001	1.17 (1.10–1.24)	<.001
Week 3	2.04	0.26 (0.14–0.38)	1.14 (1.08–1.21)	<.001	1.17 (1.10–1.25)	<.001
On ART[d] (n = 183)						
Baseline	1.76	-	-		-	-
Week 1	1.81	0.05 (-0.02–0.12)	1.02 (0.98–1.06)	.281	1.03 (0.98–1.07)	.220
Week 2	1.88	0.16 (0.07–0.26)	1.09 (1.03–1.15)	.002	1.11 (1.05–1.18)	<.001
Week 3	1.93	0.18 (0.08–0.29)	1.10 (1.04–1.17)	.002	1.13 (1.06–1.21)	<.001
Off ART[d] (n = 17)						
Baseline	2.29	-	-		-	-
Week 1	3.02	0.74 (0.27–1.20)	1.32 (1.16–1.52)	.000	1.35 (1.13–1.61)	.001
Week 2	3.56	1.22 (0.70–1.74)	1.51 (1.27–1.80)	<.001	1.57 (1.32–1.87)	<.001
Week 3	3.36	1.08 (0.54–1.62)	1.49 (1.26–1.77)	<.001	1.51 (1.26–1.80)	<.001

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; IRR, incident rate ratio.

[a]Absolute change and 95% CI of cervical log_{10} copies/swab human immunodeficiency virus (HIV) RNA in comparison to baseline.

[b]IRR and 95% CI in comparison to baseline.

[c]Adjusted for ART and detectable plasma HIV RNA.

[d]Adjusted for detectable plasma HIV RNA.

significantly increased to 3.31 log_{10} copies ($P = .011$) and 3.80 log_{10} copies ($P = .002$), respectively.

DISCUSSION

In this study we examined the impact of LEEP and cryotherapy on cervical HIV shedding. Women treated with LEEP had significantly higher mean cervical HIV RNA levels compared to those who were treated with cryotherapy for 3 weeks following treatment. Women treated with LEEP had higher cervical HIV RNA levels throughout the follow-up period compared to baseline, regardless of ART status or plasma viral load. In contrast, cervical HIV RNA levels did not increase after treatment with cryotherapy or among women on ART. Among women treated with cryotherapy, only women off ART had higher HIV RNA levels during follow-up.

Table 3. Change in Mean Cervical Human Immunodeficiency Virus RNA (log_{10} copies/swab) Among Women Treated With Cryotherapy

Time	Mean log_{10} Copies	Absolute Mean Change From Baseline (95% CI)[a]	Unadjusted IRR[b] (95% CI)	*P* Value	Adjusted IRR[b] (95% CI)	*P* Value
Overall[c] (n = 200)						
Baseline	2.02	-	-		-	-
Week 1	1.96	−0.02 (−0.11 to 0.06)	0.99 (0.95 to 1.03)	.566	0.98 (0.94–1.03)	.390
Week 2	2.04	0.07 (−0.02 to 0.16)	1.04 (0.99 to 1.09)	.150	1.04 (0.99–1.10)	.102
Week 3	2.12	0.05 (−0.05 to 0.14)	1.04 (0.99 to 1.10)	.122	1.05 (0.99–1.11)	.104
On ART[d] (n = 172)						
Baseline	1.88	-	-		-	-
Week 1	1.82	−0.02 (−0.11 to 0.06)	0.99 (0.95 to 1.03)	.627	0.98 (0.93–1.03)	.386
Week 2	1.86	0.03 (−0.06 to 0.12)	1.00 (0.96 to 1.05)	.877	1.01 (0.96–1.07)	.658
Week 3	1.86	−0.01 (−0.11 to 0.08)	0.99 (0.94 to 1.05)	.831	1.00 (0.94–1.05)	.899
Off ART[d] (n = 28)						
Baseline	2.88	-	-		-	-
Week 1	2.83	−0.04 (−0.33 to 0.26)	0.99 (0.89 to 1.09)	.812	0.99 (0.88–1.11)	.864
Week 2	3.31	0.45 (0.03 to 0.86)	1.18 (1.04 to 1.35)	.011	1.17 (1.02–1.34)	.024
Week 3	3.80	0.68 (0.18 to 1.17)	1.25 (1.08 to 1.44)	.002	1.25 (1.07–1.45)	.004

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; IRR, incident rate ratio.

[a]Absolute change and 95% CI of cervical log_{10} copies/swab human immunodeficiency virus (HIV) RNA in comparison to baseline.

[b]IRR and 95% CI of cervical log_{10} copies/swab HIV RNA in comparison to baseline.

[c]Adjusted for ART and detectable plasma HIV RNA.

[d]Adjusted for detectable plasma HIV RNA.

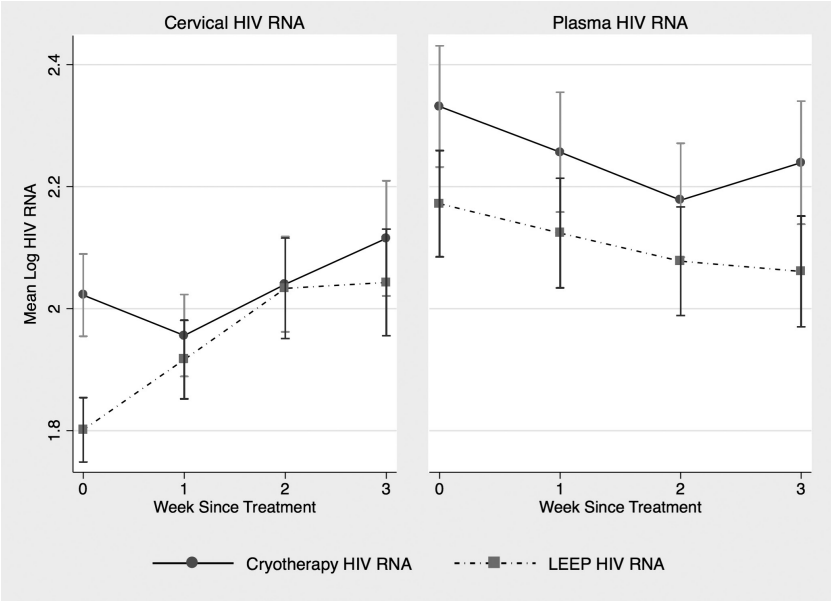

Figure 2. Weekly treatment level mean cervical and plasma human immunodeficiency virus RNA over 3 weeks of follow-up. Abbreviations: HIV, human immunodeficiency virus; LEEP, loop electrosurgical excision procedure.

The results of our study are consistent with a recent study that demonstrated that LEEP treatment resulted in a significant short-term increase in HIV shedding at 2 weeks following treatment, which returned to baseline by 4 weeks [22]. Immune activation in the genital tract recruits CD4+ T cells to the area of tissue damage, increasing HIV target cells [27]. Proinflammatory cytokines predict HIV shedding [18, 28], and higher levels of cervical HIV RNA and proinflammatory cytokines have been measured following CIN treatment [21]. As an excisional procedure, LEEP removes both the lesion and healthy cervical tissue around the lesion [22]. The subsequent ulceration that results from LEEP treatment covers a greater surface area and may thereby promote a greater inflammatory response than cryotherapy [11]. Depending on the size of the loop electrode used, tissue should be excised to a depth of 6–7 mm, but it is not uncommon for the depth of tissue removal to exceed 10 mm [4, 29]. By removing cells of both the ecto- and endocervix, LEEP may contribute to HIV shedding from the upper genital tract in addition to the lower tract [30]. In contrast, cryotherapy causes necrosis and damage to ectocervical cells by freezing tissue up to 5–7 mm deep [31]. Typical CIN2/3 lesions are <5 mm in depth from the surface [32]. Compared to LEEP, the inflammation following cryotherapy treatment may be more localized and therefore cause less HIV shedding.

The transmission risk of female-to-male HIV transmission has been shown to be 1.56-fold higher for every 1-$\log_{10}$ increase in cervical HIV RNA after adjusting for plasma HIV RNA [14]. In our study, the magnitude of change in cervical HIV RNA from baseline was <0.20 $\log_{10}$ among women on ART in both LEEP and cryotherapy cohorts. However, localized inflammation is significantly associated with increased cervical HIV shedding [33], and women on ART with undetectable plasma HIV RNA have still been found to shed HIV from the cervix [34, 35]. Women not on ART experienced a mean change of >1-log change following LEEP and 0.68 $\log_{10}$ following cryotherapy. ART appears to modulate changes in cervical HIV levels, and thus the risk of sexual HIV transmission may not pose a substantial risk for women on ART. However, there may be increased infectivity among women who are not on ART at the time of cervical treatment, particularly among those receiving LEEP.

Our study has several limitations. First, we reported an imbalance between arms in cervical HIV RNA at baseline, and we addressed this by adjusting for baseline cervical viral load. Second, the study was limited by the number of women not on ART, which decreased the precision of the study to evaluate the effect of treatment and the role of ART. While these data are informative for clinical decision-making, further research on the role of ART in mitigating post-treatment cervical HIV shedding is warranted to more effectively assess the risk of HIV transmission. We only measured cervical HIV shedding for 3 weeks after treatment and did not determine when cervical viral load returned to baseline. However, prior studies have shown that cervical HIV RNA levels return to baseline after 4 weeks [22, 23], suggesting that uncomplicated treatment results only in short-term changes to the cervix that were likely captured within the course of the study.

As access to ART continues to expand globally and HIV-infected women are living healthier and more productive lives, screening and treatment for cervical cancer may be a critical component in further extending their quality of life. The results

of our study support the current guidelines that HIV-infected women avoid sexual intercourse for 1 month after treatment to allow the wound to heal or to practice risk-reduction strategies to minimize risk of HIV transmission. Our data indicate that there is an increase in cervical HIV shedding that differs by treatment procedure and ART status and that HIV-infected women should be counseled on the risk of HIV sexual transmission. Our results suggest that cryotherapy used in resource-limited settings likely does not contribute to HIV transmission during the immediate post-procedure period and is optimally performed when women are on ART to minimize cervical shedding. LEEP treatment may contribute to a time-limited increase in HIV infectivity among women not on ART, and these changes likely occur within the 1-month interval following treatment.

Notes

Acknowledgments. We thank the research personnel, clinic and laboratory staff, and data management teams in Nairobi, Kenya, Seattle, Washington, and Lyon, France, for their work. We appreciate the Coptic Hope Center for Infectious Diseases for their cooperation. Most of all, we thank the women for participation in the study.

Funding. This research has been supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the Centers for Disease Control and Prevention (CDC) under the terms of cooperative agreements GH002036 and GH002001.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. De Vuyst H, Alemany L, Lacey C, et al. The burden of human papillomavirus infections and related diseases in sub-Saharan Africa. Vaccine **2013**; 31(Suppl 5):F32–46.
2. Harris TG, Burk RD, Palefsky JM, et al. Incidence of cervical squamous intraepithelial lesions associated with HIV serostatus, CD4 cell counts, and human papillomavirus test results. JAMA **2005**; 293:1471–6.
3. Massad LS, Einstein MH, Huh WK, et al. 2012 updated consensus guidelines for the management of cervical cancer screening test and cancer precursors. Am Soc Colposc Cerv Pathol **2013**; 17:S1–27.
4. Jordan J, Martin-Hirsch P, Arbyn M, et al. European guidelines for clinical management of abnormal cervical cytology, part 2. Cytopathology **2009**; 20:5–16.
5. Denny L, Kuhn L, De Souza M, Pollack AE, Dupree W, Wright TC Jr. Screen-and-treat approaches for cervical cancer prevention in low-resource settings: a randomized controlled trial. JAMA **2005**; 294:2173–81.
6. Goldie SJ, Gaffikin L, Goldhaber-Fiebert JD, et al; Alliance for Cervical Cancer Prevention Cost Working Group. Cost-effectiveness of cervical-cancer screening in five developing countries. N Engl J Med **2005**; 353:2158–68.
7. Sankaranarayanan R, Anorlu R, Sangwa-Lugoma G, Denny LA. Infrastructure requirements for human papillomavirus vaccination and cervical cancer screening in sub-Saharan Africa. Vaccine **2013**; 31(Suppl 5):F47–52.
8. Sankaranarayanan R, Nene BM, Dinshaw KA, et al; Osmanabad District Cervical Screening Study Group. A cluster randomized controlled trial of visual, cytology and human papillomavirus screening for cancer of the cervix in rural India. Int J Cancer **2005**; 116:617–23.
9. Lince-Deroche N, Phiri J, Michelow P, Smith JS, Firnhaber C. Costs and cost effectiveness of three approaches for cervical cancer screening among HIV-positive women in Johannesburg, South Africa. PLoS One **2015**; 10:e0141969.
10. Chamot E, Kristensen S, Stringer JS, Mwanahamuntu MH. Are treatments for cervical precancerous lesions in less-developed countries safe enough to promote scaling-up of cervical screening programs? A systematic review. BMC Womens Health **2010**; 10:11.
11. Wright TC Jr, Subbarao S, Ellerbrock TV, et al. Human immunodeficiency virus 1 expression in the female genital tract in association with cervical inflammation and ulceration. Am J Obstet Gynecol **2001**; 184:279–85.
12. Wang CC, McClelland RS, Reilly M, et al. The effect of treatment of vaginal infections on shedding of human immunodeficiency virus type 1. J Infect Dis **2001**; 183:1017–22.
13. Chirenje ZM, Rusakaniko S, Akino V, Mlingo M. A randomised clinical trial of loop electrosurgical excision procedure (LEEP) versus cryotherapy in the treatment of cervical intraepithelial neoplasia. J Obstet Gynaecol **2001**; 21:617–21.
14. Baeten JM, Kahle E, Lingappa JR, et al. Genital HIV-1 RNA quantity predicts risk of heterosexual HIV-1 transmission. Sci Transl Med **2011**; 3:1–15.
15. Launay O, Tod M, Tschöpe I, et al; ANRS EP24 GYNODYN Study Group. Residual HIV-1 RNA and HIV-1 DNA production in the genital tract reservoir of women treated with HAART: the prospective ANRS EP24 GYNODYN study. Antivir Ther **2011**; 16:843–52.
16. Mhatre M, McAndrew T, Carpenter C, Burk RD, Einstein MH, Herold BC. Cervical intraepithelial neoplasia is associated with genital tract mucosal inflammation. Sex Transm Dis **2012**; 39:591–7.
17. Johnson LF, Lewis DA. The effect of genital tract infections on HIV-1 shedding in the genital tract: a systematic review and meta-analysis. Sex Transm Dis **2008**; 35:946–59.
18. Mitchell C, Hitti J, Paul K, et al. Cervicovaginal shedding of HIV type 1 is related to genital tract inflammation independent of changes in vaginal microbiota. AIDS Res Hum Retroviruses **2011**; 27:35–9.
19. Gitau RW, Graham SM, Masese LN, et al. Effect of acquisition and treatment of cervical infections on HIV-1 shedding in women on antiretroviral therapy. AIDS **2010**; 24:2733–7.
20. Fiore JR, Suligoi B, Saracino A, et al. Correlates of HIV-1 shedding in cervicovaginal secretions and effects of antiretroviral therapies. AIDS **2003**; 17:2169–76.
21. Lawn SD, Subbarao S, Wright TC Jr, et al. Correlation between human immunodeficiency virus type 1 RNA levels in the female genital tract and immune activation associated with ulceration of the cervix. J Infect Dis **2000**; 181:1950–6.
22. Huchko MJ, Woo VG, Liegler T, et al. Impact of loop electrosurgical excision procedure for cervical intraepithelial neoplasia on HIV-1 genital shedding: a prospective cohort study. BJOG **2013**; 120:1233–9.
23. Chung MH, McKenzie KP, Richardson BA, et al. Cervical HIV-1 RNA shedding after cryotherapy among HIV-positive women with cervical intraepithelial neoplasia stage 2 or 3. AIDS **2011**; 25:1915–9.
24. Forhan SE, Godfrey CC, Watts DH, Langley CL. A systematic review of the effects of visual inspection with acetic acid, cryotherapy, and loop electrosurgical excision procedures for cervical dysplasia in HIV-infected women in low- and middle-income countries. J Acquir Immune Defic Syndr **2015**; 68(Suppl 3):S350–6.
25. Greene SA, Nyongesa-Malava E, Richardson BA, et al. Randomized trial of LEEP vs cryotherapy to treat CIN 2/3 in HIV-infected women. In: Conference on Retroviruses and Opportunistic Infections. Seattle, Washington, 2017.
26. Chung MH, Drake AL, Richardson BA, et al. Impact of prior HAART use on clinical outcomes in a large Kenyan HIV treatment program. Curr HIV Res **2009**; 7:441–6.
27. Jaspan HB, Liebenberg L, Hanekom W, et al. Immune activation in the female genital tract during HIV infection predicts mucosal CD4 depletion and HIV shedding. J Infect Dis **2011**; 204:1550–6.
28. Nkwanyana NN, Gumbi PP, Roberts L, et al. Impact of human immunodeficiency virus 1 infection and inflammation on the composition and yield of cervical mononuclear cells in the female genital tract. Immunology **2009**; 128(1 Suppl):e746–57.
29. Noehr B, Jensen A, Frederiksen K, Tabor A, Kjaer SK. Depth of cervical cone removed by loop electrosurgical excision procedure and subsequent risk of spontaneous preterm delivery. Obstet Gynecol **2009**; 114:1232–8.
30. Coleman JS, Hitti J, Bukusi EA, et al. Infectious correlates of HIV-1 shedding in the female upper and lower genital tracts. AIDS **2007**; 21:755–9.
31. Khan MJ, Smith-McCune KK. Treatment of cervical precancers: back to basics. Obstet Gynecol **2014**; 123:1339–43.
32. Abdul-Karim FW, Fu YS, Reagan JW, Wentz WB. Morphometric study of intraepithelial neoplasia of the uterine cervix. Obstet Gynecol **1982**; 60:210–4.
33. Blish CA, McClelland RS, Richardson BA, et al. Genital inflammation predicts HIV-1 shedding independent of plasma viral load and systemic inflammation. J Acquir Immune Defic Syndr **2012**; 61:436–40.
34. Neely MN, Benning L, Xu J, et al. Cervical shedding of HIV-1 RNA among women with low levels of viremia while receiving highly active antiretroviral therapy. J Acquir Immune Defic Syndr **2007**; 44:38–42.
35. Cu-Uvin S, DeLong AK, Venkatesh KK, et al. Genital tract HIV-1 RNA shedding among women with below detectable plasma viral load. AIDS **2010**; 24:2489–97.