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BRIEF REPORT

Human Immunodeficiency Virus Type 1 RNA Genital Tract Shedding After Cryotherapy for Cervical Intraepithelial Neoplasia in Western Kenya

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This prospective study of 39 women living with human immunodeficiency virus (HIV) on antiretroviral therapy in Western Kenya aimed to quantify genital tract HIV-1 RNA (GT-HIV RNA) shedding before and after cryotherapy for cervical intraepithelial neoplasia. Most GT-HIV RNA shedding was detected precryotherapy, suggesting that cryotherapy was not the primary cause of shedding.

Keywords. cervical cancer; cervical cancer treatment; cryotherapy; HIV; HIV genital tract viral load; Kenya.

Cervical cancer has the highest mortality of gynecologic cancers in Kenya [1]. The estimated prevalence of human immunodeficiency virus (HIV) in Kenya is 4.0% and majority of adult PWH in Kenya are women (63.5%) [2]. The high incidence of HIV and human papillomavirus (HPV) coinfection that could progress to cervical cancer raises the priority of this disease burden. Furthermore, people with HIV (PWH) requiring loop electrosurgical excision procedure (LEEP) or cryotherapy are typically associated with the higher-risk HPV serotypes 16 or 18 [3].

Cryotherapy is a treatment for low-grade cervical intraepithelial neoplasia (CIN) [4]. Cryotherapy causes cervical inflammation, ulcerations, and bleeding, which may theoretically increase the risk of genital tract HIV type 1 RNA (GT-HIV RNA) shedding and thereby the risk of HIV sexual transmission [5]. GT-HIV RNA is strongly correlated with sexual transmission [6]. While antiretroviral therapy (ART) is the standard of care to maximally suppress the virus, halt disease progression, and prevent transmission [7], GT-HIV RNA can differ from the plasma viral load (PVL) [8,9], and there is still heightened risk of HPV-related malignancy [5].

This study evaluated if cryotherapy increases GT-HIV RNA. Only 2 studies have evaluated the risk of increased cervical shedding after cryotherapy. Chung et al reported that ART-adherent women did not have any detectable increase of HIV RNA postcryotherapy [1]. Greene et al showed that cryotherapy is the preferred CIN treatment for PWH compared to LEEP because of significantly higher post-LEEP GT-HIV RNA compared to postcryotherapy levels, in addition to elevated GT-HIV RNA post-LEEP for both ART-naive and ART-treated PWH compared to only increased GT-HIV RNA among ART-naive PWH postcryotherapy [10]. Both studies advocated for further analysis to evaluate the effect of cryotherapy.

METHODS

This longitudinal study enrolled 52 participants at the Moi Teaching and Referral Hospital in Eldoret, Kenya, between March 2015 and June 2017. Participants were referred by the Academic Model Providing Access to Healthcare (AMPATH) Cervical Cancer Screening and Prevention Program where trained nurses screened women using visual inspection with acetic acid (VIA). Eligibility for cryotherapy was VIA-positive lesions that covered <75% of the cervix, with clear margins, no extension into the endocervix, and no satellite lesions. The study intended to enroll ART-treated and ART-naive PWH. However, the World Health Organization's treatment guidelines changed, and all PWH were started on ART regardless of CD4 count or PVL [11].

Institutional review board approvals were obtained from Moi Teaching and Referral Hospital and the Miriam Hospital. The study protocol conformed to standards of care in Kenya. Written informed consent was obtained prior to cryotherapy administration. Demographic information and a full medical history (CD4 count, plasma HIV-1 RNA levels, ART adherence, contraception, reproductive history, menstrual history, sexual history, and other medical comorbidities) were obtained by questionnaire at the initial visit. The study was offered to all PWH on ART who met the eligibility criteria of female sex, age ≥18 years, documented HIV-1 infection, undergoing VIA and cryotherapy for low-grade CIN as determined by their physicians, not pregnant, no history of pregnancy or dilation and curettage within the last 3 months,

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no current mucopurulent discharge or genital ulcer, no sexually transmitted infections (STIs) or cervical infection in the last 2 weeks, no active vaginal bleeding, generally healthy and no debilitating disease, no acute systemic illness, no systemic steroids in the past 3 months, no immunization in the past month, no use of immunomodulatory drugs except ART, on ART for at least 3 months, able to give informed consent, and agreed to procedures and follow-up visits.

At the baseline visit, TearFlo (filter paper) collections from HUB Pharmaceuticals were used to obtain secretions from the endocervix for viral load (VL) testing. GT-HIV RNA VL was tested using the Abbott RealTime HIV-1 assay on the m2000 system (Abbott Molecular) with a limit of detection of 1680 copies/mL. The assay has been validated for genital specimens such as cervicovaginal lavage and been used in prior studies to quantify HIV-1 [12]. VL obtained from the RealTime polymerase chain reaction (PCR) assay was adjusted for the fact that TearFlo input volume was 24 µL. Three TearFlo strips were used per patient and each strip could hold 8 µL of sample. The assay had internal controls to monitor for any PCR inhibition, although rare. The most recent plasma HIV-1 RNA was collected from medical records. ART adherence was self-reported by questionnaire at the initial visit. The questionnaire scored adherence as "good," "fair," or "poor" by assessing a participant's frequency of missed medications within the past 2 weeks based on barriers to medication medication-taking [13].

At baseline, all women were tested for pregnancy and STIs including syphilis by rapid plasma reagin (Omega), HSV-2 by serology (Murex), gonorrhea and chlamydia by urine PCR (Abbott), bacterial vaginosis by Gram stain (Abbott), and trichomoniasis by In Pouch culture (BioMed Diagnostics) by AMPATH reference laboratory. Any participants diagnosed with an STI were offered standard care based on the 2015 Centers for Disease Control and Prevention sexually transmitted disease treatment guidelines. Samples were not collected during menses.

Cryotherapy was performed for participants with low-grade CIN. The participants were advised not to have sex, douche, or insert any intravaginal products for at least 4–6 weeks after cryotherapy. Condoms were provided to reduce any risk of transmission. Follow-up visits took place at 2 and 8 weeks postcryotherapy. At each visit, TearFlo strips were collected for genital tract VL testing.

RESULTS

This study assessed 54 PWH for eligibility to enroll in the study, of which 2 were excluded for not meeting the eligibility criteria (Figure 1). At baseline, 52 participants were enrolled, of whom 4 had detectable GT-HIV RNA before cryotherapy was performed. At most recent PVL to baseline, 20 were virally suppressed, 10 were not virally suppressed, and 9 had no recent PVL. Thirty participants attended visit 2 at 2 weeks postcryotherapy, while 9 participants missed visit 2 but returned for visit 3. There were 26 participants at visit 3 while 13 participants missed visit 3. Overall, 9 were lost to follow-up, 2 withdrew due to relocation, and 2 died from causes unrelated to the study and excluded from analysis. Participants were likely lost to follow-up due to difficulty reaching recruited participants via phone, relocation, or the inconvenience of the short-interval follow-up schedule that was not synchronized with routine visits even despite our study compensating for transportation costs. In total, 39 participants who completed the baseline and at least 1 follow-up visit were eligible for analysis.

The median age among 39 participants was 37 (range, 18–57) years. The median years with HIV was 8 (range, 0–17). The median last CD4 count was 414 (range, 21–1045) cells/µL. The median recent plasma HIV-1 RNA was undetectable (range, undetectable to 369 794 copies/mL). The majority had no previous STI history and only 18% reported acquiring HIV by heterosexual intercourse; 82% reported unknown mode of transmission. The majority (72%) knew their current partner's HIV status; 56% reported having a sexual partner with HIV while 30% had a partner without HIV. The majority (89%) were on a combination of 2 nucleoside reverse transcriptase inhibitors (NRTIs) and a nonnucleoside reverse transcriptase inhibitor or 2 NRTIs and protease inhibitors (8.3%). Most reported "good" adherence to ART (74%). Two participants reported the use of prophylactic antibiotics for low CD4 counts. Most (72%) were monogamous and most used condoms with male sexual partners always (41%) or sometimes (18%). Median age of first sexual experience was 16 (range, 13–21) years.

Before cryotherapy, 4 of 39 (10.3%) participants had detectable GT-HIV RNA with a mean of 43 109 (range, 21 812–73 625) copies/mL (Table 1). Two weeks postcryotherapy, only 1 of 30 participants (3.3%) had a detectable GT-HIV RNA level of 73 125 copies/mL; she had no shedding precryotherapy but had a plasma HIV-1 RNA of 49 124 copies/mL 3 months after enrollment. Eight weeks postcryotherapy, 3 of 26 (11.5%) participants had detectable GT-HIV RNA and the mean GT-HIV RNA of these 3 was 44 668 (range, 21 256–64 812) copies/mL. One of the 3 had high plasma HIV-1 RNA level of 150 695 copies/mL 3 months precryotherapy. The other 2 had GT-HIV RNA shedding at baseline despite undetectable most recent plasma HIV-1 RNA level. However, their undetectable plasma HIV-1 RNA was 8–11 months precryotherapy, which may not accurately reflect PVL at baseline.

DISCUSSION

In total, 6 of the 39 participants (15.3%) had GT-HIV RNA shedding at any point during the study. Two of the 6 had recent

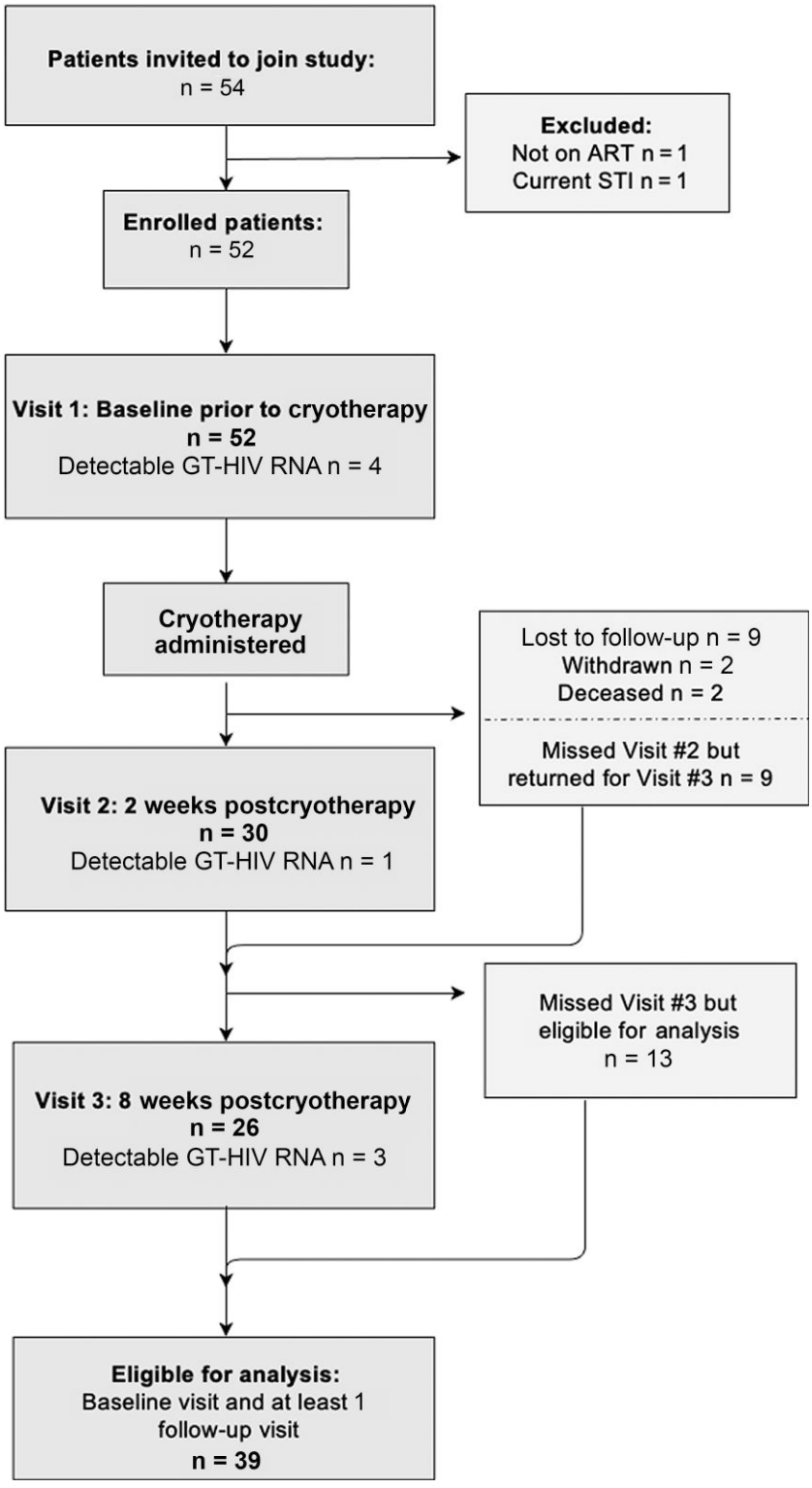

Figure 1. Enrollment and eligibility participant flow diagram. Of 54 participants invited to join the study, 2 were excluded for not being on antiretroviral therapy (ART) or current sexually transmitted infection (STI) at baseline while 52 underwent cryotherapy at the baseline visit. Subsequently, 9 participants were lost to follow-up, 2 died, and 2 withdrew from the study. The 39 who had a baseline visit and at least 1 follow-up visit were included in the analysis. Of the 39, 4 (10.3%) had detectable genital tract HIV RNA (GT-HIV RNA) before cryotherapy and 1 of 30 (3.3%) had shedding 2 weeks postcryotherapy and 3 of 26 (11.5%) had shedding 8 weeks after cryotherapy.

Table 1. Plasma and Genital Tract Human Immunodeficiency Virus (HIV) Viral Loads Before and After Cryotherapy of Participants With Detectable Genital Tract HIV RNA

Participant With Detectable GT-HIV RNA	Most Recent PVL	GT-HIV RNA Viral Load		
		Precryotherapy	2 Weeks Postcryotherapy	8 Weeks Postcryotherapy
#1	150 695 copies/mL (3 mo precryotherapy)	Undetectable	Undetectable	21 256 copies/mL
#2	49 124 copies/mL (3 mo postcryotherapy)	Undetectable	73 125 copies/mL	Undetectable
#3	Undetectable (4 mo precryotherapy)	38 625 copies/mL	Undetectable	Undetectable
#4	Undetectable (1 mo precryotherapy)	38 375 copies/mL	No sample	Undetectable
#5	Undetectable (11 mo precryotherapy)	73 625 copies/mL	No sample	64 812 copies/mL
#6	Undetectable (8 mo precryotherapy)	21 812 copies/mL	No sample	47 937 copies/mL

Abbreviations: GT-HIV RNA, genital tract human immunodeficiency virus type 1 RNA; PVL, plasma viral load.

high levels of plasma HIV-1 RNA (range, 49 124–150 695 copies/mL) within 3 months of the study and detectable GT-HIV RNA at follow-up. The other 4 had undetectable recent plasma HIV-1 RNA within 1–11 months of the study but each had detectable GT-HIV RNA precryotherapy. This suggests that cryotherapy was not the primary cause of GT-HIV RNA shedding. Plasma HIV-1 RNA level may be the reason for detectable GT-HIV RNA.

The majority (66%) with detectable GT-HIV RNA at any point also had genital tract shedding before cryotherapy. This suggests that cryotherapy was not the primary cause of GT-HIV RNA shedding. ART nonadherence might have played a major role. Self-reported adherence was not predictive of GT-HIV RNA shedding and may not correct for bias, such as recall bias or pressure to report acceptable answers.

Our results are consistent with prior studies suggesting that there is no significant increased risk of GT-HIV RNA postcryotherapy in ART-adherent participants [4,10]. While prior studies compared levels of GT-HIV RNA after cryotherapy or LEEP between ART-naive and ART-treated participants, our study offers a unique perspective because only those on ART were eligible for analysis. Thus, in an updated clinical setting where ART is standard of care for all PWH regardless of PVL, our study highlights the role of ART adherence in maintaining viral suppression of plasma and genital tract VL and therefore the importance of considering ART adherence in PWH undergoing cryotherapy. Nonetheless, we recommend further research to assess differences between ART-adherent and ART-nonadherent PWH undergoing cryotherapy for CIN.

Our study has several limitations. The small sample size limits its generalizability. Changes to ART guidelines limited the results to descriptive analysis as there was no ART-naive control group. Adherence was self-reported at baseline and could have been more variable than our results show. For future studies, we would recommend STI testing at all visits, not just at baseline. Finally, while GT-HIV RNA was collected at each visit, neither genital HIV DNA nor corresponding plasma HIV-1 RNA was collected at each visit. Thus, recent PVL was not standardized relative to baseline, nor were GT-HIV RNA levels directly comparable to plasma HIV-1 RNA or genital HIV DNA levels at each visit. In Kenya, it is standard clinically to monitor PVL once a year given resource limitations. Of note, while PVL is the strongest predictor of GT-HIV RNA, serodiscordance between the plasma and genital tract compartments can be attributed to low immune receptor expression, poor ART efficacy in the genital tract, inflammation, or STIs [14–17].

Notes

Author contributions. This project was created by E. O. O. and S. C.-U. The data were collected by E. O. O., K. M., and K. S. with support from P. M. I. and P. K. T., and K. M. is the program coordinator and manager at the Academic Model Providing Access to Healthcare (AMPATH) Oncology Institute. A. M. C. and S. S. S. ran the viral loads. A. E. B. wrote the first draft of the article with support from S. C.-U. The analysis was conducted by A. E. B. and T. D. L. with support from A. M. C. and S. C.-U. All authors reviewed and approved the final manuscript.

Patient consent. Institutional review board approvals were obtained from Moi Teaching and Referral Hospital and the Miriam Hospital. The study conformed to standards of care in Kenya. Written informed consent was obtained prior to cryotherapy administration.

Data availability. The data used to support the findings of this study are available from the corresponding author upon request.

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Potential conflicts of interest. All authors: No potential conflicts.

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