Published in final edited form as:

Sex Transm Infect. 2011 March ; 87(2): 114–117. doi:10.1136/sti.2010.045369.

Genital Ulceration Does Not Increase HIV-1 Shedding in Cervical or Vaginal Secretions of Women Taking Antiretroviral Therapy

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Abstract

Objectives—Genital ulcer disease (GUD) is associated with increased HIV-1 RNA shedding in antiretroviral therapy (ART)-naïve women. The effect of GUD on HIV-1 shedding among ART-treated women is not known. Our objective was to test the hypothesis that genital ulcerations increase genital HIV-1 RNA shedding in women receiving ART.

Methods—Eligible women initiated ART and attended monthly visits with inspection for genital lesions and collection of genital swabs. GUD cases diagnosed after ≥ 2 months on ART were included for analysis and served as their own controls. HIV-1 RNA was quantitated in specimens collected before, during, and after GUD for all cases. The lower limit of quantitation was 100 HIV-1 RNA copies/swab. Using the pre-GUD visit as the reference, we compared detection of genital HIV-1 RNA before versus during and after GUD episodes.

Results—Thirty-six women had GUD episodes after ART initiation. HIV-1 RNA was detected before, during, and after GUD in cervical secretions from 4 (11%), 1 (3%), and 6 (17%) women respectively, and in vaginal secretions from 3 (8%), 4 (11%) and 4 (11%) women respectively. After adjustment for time on ART, there was no difference in detection of cervical HIV-1 RNA before versus during GUD (aOR 0.22, 95% CI 0.04–1.23). Likewise, GUD did not increase HIV-1 detection in vaginal secretions (adjusted odds ratio [aOR] 1.32, 95% CI 0.29–5.92).

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Competing interests. None declared for all authors.

Contributors. RSM was the PI of the study; RSM, BAR, KM, NP, WJ, JNA, and JO contributed to the design of the study; SMG was the lead author for the paper; SMG and BAR performed the statistical analysis; RSM, SMG, LM, and RG supervised conduct of the study; KM and JO supervised laboratory testing; and all authors contributed to the write up.

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Conclusions—GUD did not significantly increase cervical or vaginal HIV-1 shedding. Our results suggest that ART maintains its effectiveness for genital HIV-1 suppression despite GUD episodes.

Keywords

genital ulcer; antiretroviral therapy; HIV infection; women

Introduction

Genital ulcer disease (GUD) is a common problem in HIV-1-infected women,^{1, 2} and has been associated with increased HIV-1 RNA shedding in the absence of antiretroviral therapy (ART).³ The majority of ulcers in HIV-1-infected persons are due to infection with herpes simplex virus,⁴ which has been shown to increase HIV-1 shedding in genital secretions during both clinical and subclinical reactivations.⁵

The effect of GUD on HIV-1 shedding among ART-treated women is not known. We have previously reported that the incidence of GUD increases in the first month after ART initiation, likely as a result of immune reconstitution.⁶ After this transient increase, ART may reduce the risk of GUD episodes, although risk remains elevated compared to HIV-1-seronegative women.^{1, 7} Therefore, GUD episodes could represent a period where there is increased risk for HIV-1 transmission from ART-treated women. Our objective was to test the hypothesis that genital ulcerations increase genital HIV-1 RNA shedding in women receiving ART.

Methods

This analysis is nested within a prospective cohort study following HIV-1-infected, non-pregnant Kenyan women who initiated ART with a standard Kenyan first-line regimen. Participants attended monthly clinic visits thereafter. Women with incident GUD occurring at least 2 months after ART initiation were identified as cases, and served as their own controls. Genital HIV-1 shedding before, during, and after resolution of the GUD episode was analyzed for all cases. All participants gave written informed consent. Ethical review committees of the Kenya Medical Research Institute, the University of Washington, and the Fred Hutchinson Cancer Research Center approved the study.

At treatment initiation and monthly thereafter, women were interviewed using standardized questionnaires about recent sexual behaviour, contraceptive practices, and genitourinary symptoms. A physical examination including a pelvic speculum examination was performed by an experienced physician trained in genitourinary medicine. The vulva, vagina, and cervix were inspected for genital ulcers, defined as ulcerations or vesicular lesions of the vulvar, vaginal, or cervical mucosa. Specimens of cervical and vaginal secretions were collected for laboratory diagnosis of genital infections, as described previously.⁸ Vaginal specimens for HIV-1 quantitation are collected by rolling a Dacron swabs 3 full turns against the lateral vaginal wall. Cervical specimens for HIV-1 quantitation are obtained by inserting the swab gently into the cervical os and rotating two full turns. Adherence was monitored at each visit by pill count and a validated visual analog scale.⁹ Blood was collected for syphilis serology and CD4 cell counts at baseline and every three months thereafter. Genital tract infections were treated according to World Health Organization guidelines.¹⁰ Acyclovir was provided only for typical herpetic lesions diagnosed within 48 hours of symptom onset.

Screening for genital infections included detection of yeast and *Trichomonas vaginalis* by light microscopy of vaginal saline wet preparations. Bacterial vaginosis was evaluated by Nugent scoring of a vaginal Gram stain. After Gram staining of cervical secretions, the number of polymorphonuclear leukocytes in three non-adjacent oil immersion fields was quantified. Culture for *Neisseria gonorrhoeae* was performed on modified Thayer-Martin medium. A nucleic acid amplification assay (Aptima Combo 2, Gen-Probe, San Diego, California, USA) was used to detect *N. gonorrhoeae* and *Chlamydia trachomatis*. For atypical genital ulcers, a swab of the ulcer base was cultured for *Haemophilus ducreyi*. The presence of sperm on cervical Gram stain or vaginal wet preparation was recorded. Genital samples were frozen at -70°C until shipment to Seattle on dry ice or in liquid nitrogen for HIV-1 RNA quantitation using the Gen-Probe HIV-1 viral load assay (San Diego, California, USA).¹¹ The lower limit of quantitation was 100 copies/swab.

Because the majority of RNA levels were below the limit for quantitation (100 copies/swab), the primary analysis was binomial (i.e., above versus below the threshold for quantitation). Generalized estimating equations (GEE) with a binomial link and exchangeable correlation matrix were used to compare the presence of detectable HIV-1 RNA in cervical and vaginal secretions across the three visits, with the visit before the GUD episode as the comparator visit and the visit during the GUD episode as the first indicator visit. The visit after resolution of the GUD episode was included as a second indicator visit, to determine whether HIV-1 RNA shedding returned to baseline after the episode.

All analyses included an *a priori* adjustment for time since ART initiation. Additional adjusted analyses were performed to control for confounding. Potential confounding factors considered included pill count adherence, hormonal contraceptive use, vaginal washing, menstrual cycle timing, and the presence of concurrent bacterial vaginosis, *Candida*, trichomoniasis, or cervicitis (defined as detection of *N. gonorrhoeae*, *C. trachomatis*, or ≥ 30 polymorphonuclear leukocytes per high-power field). Because our prior studies have demonstrated that genital HIV-1 RNA shedding is higher during the last week before menses,¹² we coded menstrual cycle timing as post-menstrual [1–21 days after last period], pre-menstrual [22–35 days after last period], or amenorrheic [≥ 36 days since last period]. We used two approaches to identify variables to retain in the final model: significant change across visits and significant association with the shedding outcome (both at $p < 0.10$). Data were analyzed using Stata version 9.2 (StataCorp, College Station, Texas, USA).

Results

From March 2004 through July 2008, 145 women initiated first-line ART. Of these, 36 (24.8%) were diagnosed with a genital ulcer at least 2 months after treatment initiation. Table 1 presents the baseline characteristics of these 36 women. Of note, a history of GUD was documented during pre-ART follow-up for 26 women (72%). GUD was diagnosed a median of 5 months after ART initiation. The median CD4 count at the pre-GUD visit was 241 cells/ μL . Median adherence was 100% by both pill count and visual analog scale. Ulcer location was vulvar for 32 women (89%) and cervical for 5 (14%). One woman had both vulvar and cervical ulcers. Chancroid cultures were performed for 18 atypical ulcers (50.0%); all were negative for *H. ducreyi*. Syphilis serology was positive in two women with a history of previously treated syphilis, but there was no increase in titer to indicate acute syphilis infection. Acyclovir was provided at the GUD diagnosis visit in 3 cases.

Among the 36 women with GUD episodes, HIV-1 RNA was detected before, during, and after GUD in cervical secretions from 4 (11%), 1 (3%), and 6 (17%) women respectively. Detectable HIV-1 RNA levels ranged from 100 – 20,295 copies/mL at the 4 pre-GUD visits. HIV-1 RNA was detectable in cervical secretions at the 1 GUD visit, at 13,670 copies/mL.

At the 6 visits following resolution of GUD, detectable HIV-1 RNA levels ranged from 103 – 85,553 copies/mL. HIV-1 RNA was detected before, during, and after GUD in vaginal secretions from 3 (8%), 4 (11%), and 4 (11%) women. Detectable HIV-1 RNA levels ranged from 125 – 540 copies/mL at the 3 pre-GUD visits, 100 – 8,848 copies/mL at the 4 GUD visits, and 105 – 15,193 at the 4 post-GUD visits.

Twenty-six women had no genital HIV-1 RNA detected at any timepoint. Ten women had HIV-1 RNA detected at least once: 4 in cervical secretions only, 2 in vaginal secretions only, and 4 in both. Because higher genital HIV-1 RNA levels may be more likely to lead to transmission, we examined the characteristics of the 3 women in whom high-level shedding (arbitrarily defined as >1,000 copies/mL) was detected. One woman had high-level shedding at all 3 visits and was later diagnosed with immunologic treatment failure. Her HIV RNA levels at the pre-GUD, GUD, and post-GUD visits were 20,295 copies/mL, 13,670 copies/mL, and 85,553 copies/mL in cervical secretions and 540 copies/mL, 8,848 copies/mL, and 15,193 copies/mL in vaginal secretions. One woman had high-level shedding in cervical secretions only at the pre-GUD visit, which was 1 month after ART initiation. One woman had high-level shedding in both cervical and vaginal secretions only at the post-GUD visit, when she was noted to have lower pill count adherence (88%). Of the 10 women with detectable HIV-1 RNA at any timepoint, 5 shed at more than one visit and 5 had isolated shedding at one visit (3 before GUD, 1 with GUD, and 1 after GUD).

Tables 2 and 3 present results of the GEE analysis for cervical and vaginal HIV-1 RNA detection, respectively. There was no difference in detection of HIV-1 RNA before versus during GUD (adjusted odds ratio [aOR] 0.22, 95% confidence interval [CI] 0.04 – 1.23 for cervical secretions; aOR 1.32, 95% CI 0.29 – 5.92 for vaginal secretions). In addition, genital HIV-1 RNA detection did not differ significantly when comparing the pre-GUD visit to the visit after GUD resolution (aOR 1.49, 95% CI 0.45 – 4.91 for cervical secretions; aOR 1.23, 95% CI 0.28 – 5.52 for vaginal secretions). Results were unchanged after additional adjustment for potential confounding factors. Likewise, the results remained the same in a sensitivity analysis that excluded three visits on which sperm was detected, and in a second sensitivity analysis excluding GUD cases diagnosed within 3 months after ART initiation (data not shown). There were no associations between prior GUD history or ulcer location and HIV-1 RNA detection in cervical or vaginal secretions.

Discussion

In this study comparing genital HIV-1 RNA levels before, during, and after a GUD episode, we found that GUD did not significantly increase cervical or vaginal HIV-1 RNA detection among women on ART. High-level genital HIV-1 shedding was detected in a small number of women with incomplete viral suppression due to recent ART initiation, lower adherence, or developing treatment failure. However, GUD episodes in these women were not associated with an increase in HIV-1 levels.

Strengths of this study include prospective collection of genital swab specimens at monthly visits, prospective identification of cases, and use of visits before and after GUD from each woman as controls. This nested design enabled us to directly compare genital HIV-1 shedding before, during, and after incident GUD among the 36 women who experienced GUD after ART initiation. Our study was limited by a lack of specific diagnostic testing for ulcer aetiology. Although we did culture material from atypical ulcers for chancroid, we identified no chancroid-related GUD; this is consistent with the current low incidence of chancroid in our cohort (no cases since 1999, unpublished data) and in East Africa in general.^{13,14} Although syphilis serology can be falsely negative among HIV-infected persons with advanced immunosuppression, available serologic results from quarterly

testing of these women after the post-GUD visit reveal no cases of late seroconversion. Finally, although HSV-2 serostatus was unknown for the participants in this study, previous studies in this cohort have found HSV-2 seropositivity to be nearly universal among HIV-1-infected participants.¹⁵ Therefore, the most likely aetiology for genital ulcers in these women is probably HSV-2 reactivation.

Additional limitations of this study include the lack of plasma HIV-1 RNA assessments, which did not allow us to directly correlate detectable genital shedding with plasma HIV-1 RNA levels at the same timepoint. However, our primary objective was to determine whether genital HIV-1 RNA levels were increased in the setting of GUD. Our sample size was calculated based on the assumption that most participants would have detectable genital HIV-1 RNA with a GUD episode, which would have allowed comparisons of mean HIV-1 RNA levels at each time point. Instead, most women actually had undetectable vaginal HIV-1 RNA, and we therefore used a binomial outcome for HIV-1 RNA detection above the threshold for linear quantitation. Although this change in outcome measure led to a loss of power, our results still attest to the potency of ART for genital HIV-1 RNA suppression during therapy.

In this study, direct swabs of the ulcer base were not tested, as no comparable sample exists before or after GUD. It would be useful to directly assess viral shedding from the ulcer base in future studies, as direct contact with a vulvar lesion shedding HIV-1 RNA could lead to an increased transmission risk that is not reflected by analysis of cervical and vaginal secretions. Because unprotected intercourse involves exposure to cervical and vaginal secretions regardless of whether a genital ulcer is present, we feel these results are nonetheless helpful for informing our understanding of the additional risk of HIV-1 exposure in the setting of GUD among women on ART.

Our findings add to the evidence that increases in HIV-1 shedding related to genital mucosal conditions may be less pronounced after ART initiation. In a randomized trial evaluating the impact of suppressive herpes therapy on genital HIV-1 shedding among women taking ART, valacyclovir had no significant impact on the frequency or quantity of genital HIV-1 RNA detected in the intervention arm.¹⁶ In a secondary analysis restricted to 13 women who shed HIV-1 at least once in the baseline phase, valacyclovir reduced both the proportion of visits with detectable HIV-1 shedding and the quantity of genital HIV-1 RNA during these visits.¹⁶ This suggests that although some women may still have increased HIV-1 shedding attributable to HSV-2, the majority of women taking ART can achieve genital viral suppression despite HSV-2 reactivations.

In conclusion, GUD was not an important cause of increased genital HIV-1 shedding in a population of women with high antiretroviral adherence. ART may play an important role in reducing the risk of HIV-1 transmission even in a population with a high incidence of GUD. Future research evaluating changes in HIV-1 proviral DNA levels, a marker for shedding of HIV-1-infected cells, in the setting of incident GUD will help to further clarify whether this condition may lead to an increase in transmission risk among women taking ART.

Acknowledgments

We thank the research staff in Mombasa for their hard work, the Mombasa Municipal Council for use of clinical space, Coast Provincial General Hospital for provision of laboratory space, and the Director of the Kenya Medical Research Institute for permission to publish this paper. Special thanks go to the women who participated in this study.

Funding. Supported by funding from National Institutes of Health, grants R01 AI058698 and K23 AI69990 (SMG). Additional support for the Mombasa Field Site was received from the University of Washington Center for AIDS Research (CFAR), an NIH-funded program (P30-AI027757) which is supported by the following NIH Institutes

and Centers (NIAID, NCI, NIMH, NIDA, NICHD, NHLBI, NCCAM). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

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Table 1

Characteristics of 36 Women on Antiretroviral Therapy at Pre-GUD Visit

Characteristic	N (%) or Median (IQR)
Age, years	36 (31 – 41)
Contraceptive method	
None	26 (72.2)
Oral contraceptive pill	1 (2.8)
DMPA	9 (25.0)
History of GUD	26 (72.2)
Days since ART initiation	154 (58 – 656)
CD4 count at ART initiation	125 (76 – 168)
CD4 count at pre-GUD visit	241 (158 – 312)
Adherence	
% by visual analog scale*	100 (100 – 100)
% by pill count	100 (98.3 – 100)
Late refill (>48 hours without ART)	1 (2.8)
ART regimen	
Stavudine/lamivudine/nevirapine	34 (94.4)
Zidovudine/lamivudine/nevirapine	1 (2.8)
Stavudine/lamivudine/abacavir [†]	1 (2.8)

ART = antiretroviral therapy, DMPA = depo-medroxyprogesterone acetate, GUD = genital ulcer disease

* Three women had missing visual analog scale data at all 3 visits.

[†] One woman was on a regimen with 3 non-nucleoside reverse transcriptase inhibitors due to concurrent TB treatment.

Table 2

GEE Analysis of Cervical HIV-1 RNA Shedding >100 copies/mL

Variable	Adjusted OR[*] (95% CI), P value	Adjusted OR[†] (95% CI), P value
Pre-GUD Visit	1.0	1.0
GUD Visit	0.22 (0.04–1.23), p=0.08	0.24 (0.02–2.99), p=0.27
Post-GUD Visit	1.49 (0.45–4.91), p=0.51	2.72 (0.49–14.92), p=0.25

GEE = generalized estimating equations, OR = odds ratio

* Adjusted for duration on treatment.

[†] Adjusted for menstrual status, hormonal contraception, and duration on treatment. Menstrual status changed significantly when pre-GUD and post-GUD visits were compared ($p = 0.02$), while oral contraceptive pills were associated with a greater odds of cervical HIV-1 RNA detection on univariate analysis ($p < 0.001$) and in the final model ($p = 0.003$).

Table 3

GEE Analysis of Vaginal HIV-1 RNA Shedding >100 copies/mL

Variable	Adjusted OR[*] (95% CI), P value	Adjusted OR[†] (95% CI), P value
Pre-GUD Visit	1.0	1.0
GUD Visit	1.32 (0.29–5.92), p=0.72	2.15 (0.37–12.67), p=0.40
Post-GUD Visit	1.23 (0.28–5.52), p=0.78	2.22 (0.50–9.87), p=0.30

GEE = generalized estimating equations, OR = odds ratio

* Adjusted for duration on treatment.

† Adjusted for menstrual status and duration on treatment. Menstrual status changed significantly when pre-GUD and post-GUD visits were compared (p = 0.02). No other factors were associated with vaginal HIV-1 RNA detection on univariate analysis.