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Effect of Acquisition and Treatment of Cervical Infections on HIV-1 Shedding in Women on Antiretroviral Therapy

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Abstract

Background—Cervicitis increases the quantity of HIV-1 RNA in cervical secretions when women are not taking antiretroviral therapy (ART), and successful treatment of cervicitis reduces HIV-1 shedding in this setting.

Objective—To determine the effect of acquisition and treatment of cervical infections on genital HIV-1 shedding in women receiving ART.

Design—Prospective cohort study.

Methods—We followed 147 women on ART monthly for incident non-specific cervicitis, gonorrhea, and chlamydia. Cervical swabs for HIV-1 RNA quantitation were collected at every visit. The lower limit for linear quantitation was 100 copies/swab. We compared the prevalence of HIV-1 RNA detection before (baseline) versus during and after treatment of cervical infections.

Results—Thirty women contributed a total of 31 successfully treated episodes of non-specific cervicitis (N=13), gonorrhea (N=17), and chlamydia (N=1). HIV-1 RNA was detected in cervical secretions before, during, and after cervicitis at 1 (3.2%), 5 (16.1%), and 3 (9.7%) visits respectively. Compared to baseline, detection of HIV-1 RNA was increased when cervical infections were present (adjusted odds ratio 5.7, 95% confidence interval 1.0–30.3, P=0.04). However, even in the subset of women with cervical HIV-1 RNA levels above the threshold for quantitation, most had low concentrations during cervical infections (median 115, range 100–820 copies/swab).

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Conclusions—While these data show a statistically significant increase in cervical HIV-1 RNA detection when cervical infections are present, most cervical HIV-1 RNA concentrations were near the threshold for detection, suggesting that infectivity remains low. Antiretroviral therapy appears to limit increases in genital HIV-1 shedding caused by cervical infections.

Keywords

Cervical infection; HIV-1 Shedding; Antiretroviral therapy; Women; Africa

INTRODUCTION

Heterosexual contact is the predominant mode of human immunodeficiency virus type 1 (HIV-1) transmission in Africa (1). The quantity of HIV-1 in genital secretions is likely to play an important role in mediating transmission risk (2–4). In women, ART rapidly reduces genital HIV-1 shedding (3,5). Nonetheless, up to 15% of women on ART continue to have detectable levels of HIV-1 in genital secretions (3,5–7). The reasons for continued genital HIV-1 shedding among women on ART have not been fully characterized.

Genital infections have been identified as a significant determinant of genital HIV-1 shedding when women are not on ART (8–10). Conversely, treatment of genital infections reduces the concentration of HIV-1 RNA in genital secretions. For example, in a cohort of ART-naïve women, treatment of cervical infections significantly reduced cervical HIV-1 RNA shedding (11).

With increasing interest in the use of ART to reduce HIV-1 transmission, it will be important to understand the effect of genital infections on HIV-1 shedding. This study examined the effects of cervical infections and their treatment on genital HIV-1 RNA shedding in women receiving ART.

METHODS

Population and Procedures

HIV-1-seropositive women in the Mombasa Cohort and eligible for ART were invited to participate in a prospective study of risk factors for genital HIV-1 shedding. Detailed procedures have been described previously (3). Briefly, eligible women were counseled on adherence and side effects before initiating treatment with stavudine or zidovudine, lamivudine, and nevirapine according to Kenyan guidelines (12).

At monthly visits, women completed an interview using standardized questionnaires, a physical examination was performed, and samples collected for laboratory diagnosis of genital infections and HIV-1 quantitation. Cervical specimens for HIV-1 quantitation were collected first, by inserting a Dacron swab in the cervical os and rotating two full turns. Swabs for Gram stain and culture for *Neisseria gonorrhoeae* were collected next. Every three months, an additional cervical swab was collected for detection of *N. gonorrhoeae* and *Chlamydia trachomatis* by nucleic acid amplification. Blood samples for CD4 lymphocyte count were collected every three months.

Women were identified as cases if they had incident cervical infections at least one month after initiating ART. Each woman served as her own control, with visits before infection and after successful treatment. Symptomatic genital infections were treated syndromically in accordance with Kenyan and World Health Organization guidelines (13). In addition, women were asked to return for laboratory results one week after each examination. At this visit, additional treatment was dispensed if indicated. *Neisseria gonorrhoeae* was treated

with 800mg of norfloxacin as a single dose. *Chlamydia trachomatis* was treated with 100mg of doxycycline twice daily for seven days. Syndromic treatment for nonspecific cervicitis included norfloxacin plus doxycycline. Participants underwent a repeat examination at a test-of-cure visit after one month.

Trained study nurses provided free condoms and HIV-1 risk reduction counseling at each visit. Ethical review boards from the University of Washington and Kenya Medical Research Institute approved the study. All participants provided written informed consent.

Laboratory Methods

Human Immunodeficiency Virus Type-1 screening was performed using ELISA (Detect HIV1/2, BioChem Immunosystems, Montreal Canada). Positive results were confirmed with a second ELISA (Recombigen, Cambridge Biotech, Worchester, MA, USA or Vironostika HIV-1 Uniform 11 AG/AB, bioMerieux, Macy l'Etoile, France). Quantitation of CD4 lymphocytes was performed by FACSCount (Becton Dickinson, Forest Lakes, NJ).

Non-specific cervicitis was defined as an average of ≥30 polymorphonuclear leukocytes in three non-adjacent oil immersion fields on cervical gram stains. Endocervical secretions were inoculated on modified Thayer-Martin media for isolation of *N. gonorrhoeae.* A transcription-mediated nucleic acid amplification assay (Aptima Combo2®, Gen Probe, San Diego, USA) was performed on cervical swabs for detection of *N. gonorrhoeae* and *C. trachomatis*.

Cervical swabs for HIV-1 RNA quantitation were stored in freezing media at −80°C, then shipped to Seattle on dry ice. HIV-1 RNA quantitation was performed using the Gen Probe HIV-1 viral load assay (San Diego, California, USA). The lower limit of linear quantitation was 100 copies/mL, which corresponded to 100 copies/swab, as swabs were placed in 1 mL of freezing medium.

Statistical Methods

Most women had HIV-1 RNA levels below the limit of linear quantitation. Therefore, we used generalized estimating equations with a binomial outcome distribution, logit link, and exchangeable correlation matrix to compare the presence of detectable HIV-1 RNA (above vs. below the threshold for quantitation). The pre-cervical infection visit was compared to visits during infection and after successful treatment. The primary cervicitis endpoint combined *N. gonorrhoeae, C. trachomatis,* and nonspecific cervicitis. We included visits with concurrent vaginal infections, since these have not affected cervical HIV-1 shedding in our earlier studies (9,11).

Potential confounding factors were identified as variables that differed significantly between visits or were significantly related to the outcome ($p<0.10$). Our analysis plan included *a priori* adjustment for number of months since ART initiation, as longer duration of treatment may result in greater HIV-1 suppression. The final multivariate model included hormonal contraceptive use, menstrual cycle stage, and duration on ART. Analyses were performed using SPSS 16.0 (SPSS Inc., Chicago) and Stata 9 (StataCorp College Station, TX).

RESULTS

Of 147 women on ART followed between March 2004 and December 2008, 30 participants contributed a total of 31 successfully treated episodes of cervicitis. These included 13 (41.9%) with nonspecific cervicitis, 17 (54.8%) with *N. gonorrhoeae,* and 1 (3.2%) with *C. trachomatis.* One woman contributed two infections (one episode of *C. trachomatis* and one

of *N. gonorrhoeae*). The median age of the participants was 36 years (interquartile range [IQR] 31–38). Oral contraceptive pills (N=1, 3.2%) and depot medroxyprogesterone acetate (N=7, 22.6%) were used by a minority of participants. The median duration on ART was 15 months (IQR 8–21) and participants had high adherence by pill count (median 100%, IQR 96.5–100). Twenty-nine (96.7%) were taking the standard first-line ART regimen. One woman (3.3%) was on a second-line regimen including a protease inhibitor. The median CD4 lymphocyte count at the pre-infection visit was 308 cells/μL (IQR 238–395).

HIV-1 RNA concentrations above 100 copies/swab were detected in cervical secretions before, during, and after cervicitis at 1 (3.2%), 5 (16.1%), and 3 (9.7%) visits respectively. Compared to baseline, there was a trend ($P<0.10$) for increased HIV-1 RNA detection with cervical infections (Table 1). This result was similar, but became statistically significant, after additional adjustment for contraceptive use and menstrual cycle stage (adjusted odds ratio 5.7, 95% Confidence Interval 1.0–30.3, P=0.04). Following successful treatment of cervicitis, detection of HIV-1 RNA was less frequent, and no longer significantly increased relative to baseline.

Figure 1 presents HIV-1 RNA levels by visit among women with detectable cervical shedding. The one sample with detectable HIV-1 RNA at the pre-infection visit had 407 copies/swab. At the infection visit, 5 women had detectable HIV-1 RNA (median 115, range 100–820 copies/swab). Following successful treatment, the median HIV-1 RNA level was 657 copies/swab (range 110–1770 copies/swab) in the 3 women with detectable virus. Detection of 1770 copies/swab at a post-treatment visit was the highest level of HIV-1 shedding observed. Reported pill-count adherence for this visit was 95.3%, so the reason for increased genital HIV-1 shedding was not immediately evident. We cannot rule out explanations such as measurement error rather than a true biological effect.

DISCUSSION

This study demonstrated that cervical infections may increase detection of HIV-1 RNA in cervical secretions. However, even when HIV-1 RNA was detected, most cervical HIV-1 RNA concentrations remained near the threshold for quantitation (100 copies/swab). Higher levels of HIV-1 RNA shedding are generally seen in ART-naïve women even in the absence of cervical infections. For example, in an earlier study of cervicitis among untreated HIV-1-seropositive women, median cervical HIV-1 RNA was 11,220 copies/swab at diagnosis. This was reduced to a median of 1,738 HIV-1 RNA copies/swab after successful treatment (11). In contrast, the present study of ART-treated women found that the majority have undetectable cervical HIV-1 RNA even in the presence of cervical infections.

While, acquisition of a cervical infection was associated with a statistically significant increase in cervical HIV-1 RNA, it is interesting to note that the prevalence of detectable cervical HIV-1 RNA at the final visit did not return to the pre-cervicitis baseline. This may simply reflect variation in detection in a study with a modest sample size, or could represent a gradual decline in genital HIV-1 shedding following infection. A similar finding has been observed in men treated for urethritis (14), with progressive reductions in seminal HIV-1 at 1 and 2 weeks post-treatment. At completion of follow-up, seminal HIV-1 RNA remained higher than in a control group without urethritis.

Our study used genital HIV-1 RNA as a surrogate marker for infectivity. Recent studies among HIV-1-serodiscordant couples have demonstrated that higher genital HIV-1 levels are associated with increased transmission risk (15). This association was present even after adjustment for plasma HIV-1 RNA concentration, suggesting that genital HIV-1 RNA level is a useful surrogate marker for infectivity.

Recently, there has been interest in the use of ART to reduce HIV-1 transmission. A systematic review found that the overall risk of transmission was reduced by 92% in HIV-1-serodiscordant couples on ART compared to couples in which the index case was untreated (16). Our results further highlight the potential benefits of ART as a prevention strategy. Nonetheless, it should be noted that even low concentrations of genital HIV-1 RNA could present some risk of transmission.

This study had several strengths. Women were followed prospectively. Therefore, it was possible to compare genital HIV-1 RNA concentrations before, during, and after successful treatment for cervicitis. With prolonged follow-up of this cohort, we accrued 31 cervical infections for analysis. High ART adherence provided an opportunity to determine the effect of cervicitis on HIV-1 shedding under near-optimal conditions. With lower ART adherence, cervicitis could have a greater impact on genital HIV-1 shedding.

There were limitations to this study. Because of the modest sample size, we did not have adequate power to evaluate each cervical infection separately. Cervical HIV-1 RNA was below the limit for quantitation at 71% of visits, limiting the power to detect changes in cervical HIV-1 shedding. This study did not evaluate shedding of cell-associated HIV-1 proviral DNA, which may provide a better measure of the potential for cell-cell transmission. Risk factors for HIV-1 RNA and proviral DNA shedding may differ (17), and it is not known which of these markers is most closely associated with transmission risk (2).

In conclusion, even in the setting of cervicitis, cervical HIV-1 RNA concentrations remain low in the majority of women who are adherent to ART. Nonetheless, increases in cervical HIV-1 RNA occur in a minority of women during cervicitis, and could increase transmission risk. Identification and treatment of cervical infections may help to optimize the secondary HIV-1 prevention benefits of ART.

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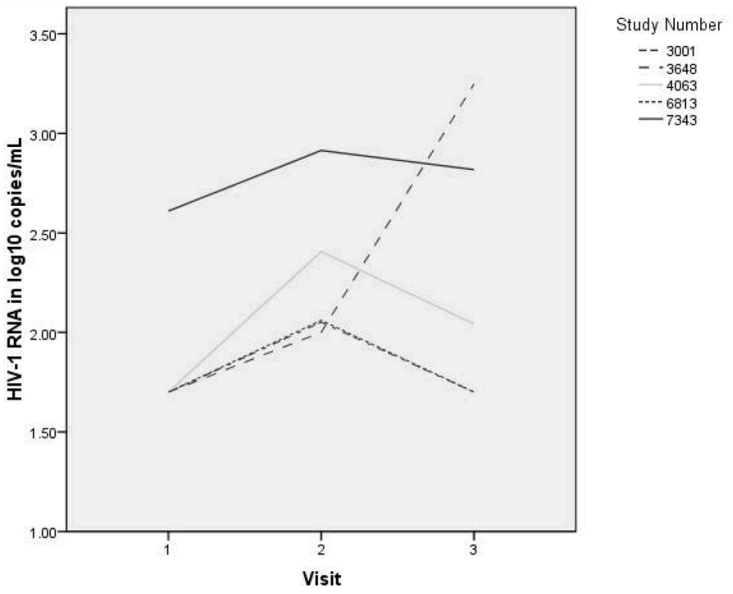

Figure 1. Log_{10} Cervical HIV-1 RNA Concentration Before, During, and After Cervicitis in the Subset of Five Women with Any Detectable Cervical HIV-1 RNA Shedding

The figure graphs log_{10}-transformed quantities of HIV-1 RNA at the pre-cervical infection, cervical infection, and post cervical infection visits in the subgroup of 5 women who had cervical HIV-1 RNA above the threshold for linear quantitation (100 copies/swab = 2.00 log_{10} copies/swab) at any time point. Cervical HIV-1 RNA concentrations below this threshold were assigned a value of half the lower limit for linear quantitation (50 copies/swab = 1.70 log_{10} copies/swab). Two participants 3001 and 6813 had near identical patterns of shedding and are superimposed on one another.

Table 1

Cervical HIV-1 RNA Detection Before, During, and After Cervical Infections

	Total Detected (%) (N=31)	aOR[a] (95% CI) (P-value)	aOR[b] (95% CI) (P-value)
Pre-Cervicitis Visit	1 (3.2%)	1.0 (reference) 6.08 (0.92–40.19)	1.0 (reference) 5.69 (1.0–30.27)
Cervicitis Visit	5 (16.1%)	(p = 0.06) 3.56 (0.59–21.56)	(p = 0.04) 2.92 (0.60–14.30)
Post-Cervicitis Visit	3 (9.7%)	(p = 0.17)	(p = 0.19)

aOR, adjusted odds ratio; 95% CI, 95% confidence interval

[a] Adjusted for duration on treatment

[b] Adjusted for hormonal contraceptive use (yes/no), week of menstrual cycle, and duration on treatment