Treatment of cervicitis is associated with decreased cervical shedding of HIV-1

R. Scott McClelland\textsuperscript{a}, Chia C. Wang\textsuperscript{a}, Kishorchandra Mandaliya\textsuperscript{b}, Julie Overbaugh\textsuperscript{c}, Maureen T. Reiner\textsuperscript{d}, Dana D. Panteleeff\textsuperscript{c}, Ludo Lavreyse\textsuperscript{e}, Jeckoniah Ndinya-Achola\textsuperscript{f}, Job J. Bwayo\textsuperscript{f} and Joan K. Kreiss\textsuperscript{a,e}

\textbf{Objective:} To determine whether cervical mucosal shedding of HIV-1 RNA and HIV-1 infected cells decreases following successful treatment of cervicitis.

\textbf{Design:} Prospective interventional study.

\textbf{Setting:} Sexually Transmitted Infections Clinic, Coast Provincial General Hospital, Mombasa, Kenya.

\textbf{Participants:} Thirty-six HIV-1 seropositive women with cervicitis: 16 with \textit{Neisseria gonorrhoeae}, seven with \textit{Chlamydia trachomatis}, and 13 with non-specific cervicitis.

\textbf{Interventions:} Treatment of cervicitis.

\textbf{Main outcome measures:} Levels of total (cell-free and cell-associated) HIV-1 RNA and presence of HIV-1 DNA (a marker for infected cells) in cervical secretions before and after resolution of cervicitis.

\textbf{Results:} After treatment of cervicitis, the median HIV-1 RNA concentration in cervical secretions was reduced from 4.05 to 3.24 log\textsubscript{10} copies/swab ($P \leq 0.001$). Significant decreases in cervical HIV-1 RNA occurred in the subgroups with \textit{N. gonorrhoeae} (3.94 to 3.28 log\textsubscript{10} copies/swab; $P = 0.02$) and \textit{C. trachomatis} (4.21 to 3.19 log\textsubscript{10} copies/swab; $P = 0.02$). Overall, the prevalence of HIV-1 infected cells in cervical secretions also decreased after treatment, from 67\% to 42\% (odds ratio, 2.8; 95\% confidence interval, 1.3–6.0; $P = 0.009$). Detection of infected cells was associated with higher mean HIV-1 RNA levels (4.04 versus 2.99 log\textsubscript{10} copies/swab; $P < 0.0001$).

\textbf{Conclusions:} Effective treatment of cervicitis resulted in significant decreases in shedding of HIV-1 virus and infected cells in cervical secretions. Treatment of sexually transmitted diseases may be an important means of decreasing the infectivity of HIV-1 seropositive women by reducing exposure to HIV-1 in genital secretions.

\copyright 2001 Lippincott Williams & Wilkins

\textit{AIDS} 2001, 15:105–110

\textbf{Keywords:} HIV-1, cervical HIV shedding, Africa, sexually transmitted diseases, infectivity, HIV transmission

\textbf{Introduction}

The epidemic of HIV-1 continues to spread rapidly, particularly in developing countries. In 1999, there were 5.4 million new infections worldwide, with more than 70\% occurring in sub-Saharan Africa [1]. In the face of the continuing and rapid spread of HIV-1, attention has been focused on the identification of...
modifiable risk factors associated with transmission of the virus. Sexually transmitted diseases (STDs) may facilitate transmission, and are highly prevalent among many populations in which HIV-1 infection is common [2,3]. Thus, there has been interest in evaluating interventions to decrease the prevalence of STDs as a means of controlling the spread of HIV-1. One such intervention, a randomized community trial of STD treatment, demonstrated a 42% reduction in HIV-1 incidence over 2 years [4]. At least two potential mechanisms could explain this decreased incidence. First, treatment of STDs in HIV-1 seronegative individuals might decrease their susceptibility to HIV-1 infection. Second, the infectivity of HIV-1 positive individuals might be reduced with treatment of co-existing STDs. If this second mechanism is important in female-to-male transmission, a decrease in genital mucosal shedding of HIV-1 might be seen with resolution of cervical or vaginal infections.

The link between STDs or genital tract inflammation and shedding of HIV-1 has been suggested in previous studies. Cross-sectional investigations have established that detection of HIV-1 in the genital tract of both women [5–9] and men [10,11] is increased in the presence of inflammation or STDs. Intervventional studies in men have shown a decrease in shedding of HIV-1 RNA in seminal plasma [12] as well as proviral DNA in urethral secretions [13] after treatment of urethritis. One study in women found that successful treatment of Neisseria gonorrhoeae significantly reduced the frequency of detection of HIV-1 RNA in cervicovaginal lavage fluid [14]. No studies have evaluated changes in cervical site-specific shedding or quantitative changes in HIV-1 RNA with treatment of cervicitis.

A study was conducted among women in Mombasa, Kenya to determine the effect of successful treatment of cervicitis on the quantity of HIV-1 RNA (cell-free and cell-associated), as well as the prevalence of HIV-1 infected cells (detected by HIV-1 proviral DNA) in cervical secretions. The relationship between detection of HIV-1 infected cells and the total HIV-1 RNA level was also examined.

Materials and methods

Study population and procedures

Between September 1996 and May 1999, women presenting to the Sexually Transmitted Infections Clinic at Coast Provincial General Hospital in Mombasa, Kenya were offered confidential HIV-1 counseling and testing. Women were also referred from the maternity ward and family planning clinic. After informed consent, patients were screened for HIV-1 using a rapid serological test (Capillus HIV-1/HIV-2, Cambridge Biotech Ltd., Galway, Ireland). Samples found to be HIV-1 positive on the Capillus test were confirmed with a commercially available enzyme immunoassay (EIA; Detect, BioChem Immunosystems, Montreal, Canada). Patients testing positive were invited to participate in the study, and informed consent was obtained. The protocol was approved by the institutional review boards of the University of Nairobi and the University of Washington.

Patients were interviewed using a standardized questionnaire regarding demographic, sexual, obstetric, and medical history. Physical examination was performed and genital tract specimens were collected for diagnosis of STDs and HIV-1 detection. Ten ml of EDTA anticoagulated blood was collected for confirmation of HIV-1 serostatus and CD4 cell count. All examinations and sample collection were performed by one of two investigators (S. M. or C. W.).

Endocervical secretions were collected by inserting a dacron swab 1 cm into the cervical os and rotating it three times. The first swab was placed in a dry cryovial for detection of HIV-1 DNA. The second and third swabs were collected for HIV-1 RNA quantitation. Each was placed in 1 ml of freezing medium (70% RPMI, 20% fetal calf serum, 10% dimethylsulfoxide, with added penicillin, streptomycin, and amphotericin B). The same order was used for all sample collections. Swabs were stored on ice for up to 4 h prior to transfer to −70 °C (dry swabs) or liquid nitrogen (swabs in freezing medium). After collection of swabs for HIV-1 detection, cervical secretions were collected for Gram stain, N. gonorrhoeae culture, and Chlamydia trachomatis antigen detection. Vaginal secretions were collected for Gram stain and wet-mount.

Patients received empiric treatment for cervicitis if the cervix appeared erythematous or had purulent discharge. Treatment included ciprofloxacin 500 mg as a single dose plus doxycycline 100 mg twice a day for 7 days, or amoxicillin/clavulanic acid 500 mg, amoxicillin 3 g, and probenecid 1 g as a single dose plus erythromycin 500 mg four times a day for 7 days. The choice of medications was based on availability as well as contraindications to some medications during pregnancy and lactation. If significant cervical motion or adnexal tenderness was present, patients were treated for pelvic inflammatory disease by adding metronidazole 400 mg twice a day for 10 days. Participants were asked to return after 2–4 days for results. At that time, patients with a laboratory diagnosis of N. gonorrhoeae, C. trachomatis, or non-specific cervicitis were treated if they had not received empiric therapy. Patients were asked to return for a follow-up examination 2 weeks after treatment. A brief history, physical examination, and collection of specimens were repeated. As before, patients with cervical inflammation or purulent dis-
charge on examination were treated empirically, and all patients were asked to return after 2–4 days for results. Patients with persistent infection received treatment with an alternative regimen as described above, or with 2 g intramuscular spectinomycin plus erythromycin 500 mg four times a day for 7 days. Only patients with persistent infection were asked to return for an additional (second) follow-up examination, 2 weeks after re-treatment. The procedure at the second follow-up was identical to the first.

Baseline visits at which there was a diagnosis of N. gonorrhoeae, C. trachomatis, or non-specific cervicitis were identified. The follow-up visit for comparison was the first visit after the baseline at which there was no detection of N. gonorrhoeae, C. trachomatis, or non-specific cervicitis. Patients with bacterial vaginosis, vaginal yeast infection, and Trichomonas vaginalis were included in the analysis. In our previous study, vaginal infections were not associated with detection of cervical HIV-1 [8].

**Microbiology**

Lymphocyte subsets were counted using a manual method (Cytosphere; Coulter, Hialeah, Florida, USA) until September 1998, after which a semi-automated system (Zymmune; Bartels, Issaquah, Washington, USA) was used.

The number of polymorphonuclear leukocytes (PMNLs) in three non-adjacent high power fields on microscopy of cervical Gram stains was quantified. Culture for N. gonorrhoeae was performed on modified Thayer-Martin media. Presence of C. trachomatis was determined by EIA (MicroTrak II; Behring Diagnostics, Cupertino, California, USA). Cervicitis was defined as an average cervical PMNL count of 30 or greater, or a positive test for N. gonorrhoeae or C. trachomatis. Patients meeting the PMNL criterion, but without N. gonorrhoeae or C. trachomatis, were considered to have non-specific cervicitis.

Gram stained vaginal secretions were scored for bacterial vaginosis [15]. A vaginal wet-mount was examined for yeast and T. vaginalis.

**Detection and quantitation of HIV-1 RNA and DNA**

The quantity of HIV-1 RNA in cervical swabs was measured using the Gen-Probe quantitative HIV-1 assay (Gen-Probe Incorporated, San Diego, California, USA), as described previously for measuring plasma viral load [16]. This assay has been shown to be sensitive and specific for quantitation of RNA from the HIV-1 subtypes common in Kenya [17], predominantly clades A, C, and D [16]. The assay has been modified for use with cervical swabs in freezing media, and was demonstrated to be effective as a quantitative HIV-1 RNA assay in these samples (D. DeVange Panteleeff, S. Emery, B. A. Richardson, S. Bodrug, J. K. Kreiss and J. Overbaugh, unpublished data). Briefly, known quantities of cell-free HIV-1 from three Kenyan primary isolates (one each of clades A, C, and D) [17] were spiked into freezing medium alone, freezing medium with a dacron swab, or freezing medium with a dacron swab plus uninfected human cells. The same quantities of HIV-1 were also added to uninfected plasma as described previously [17]. The mean values from eight replicate tests in freezing medium alone, freezing medium with a dacron swab, and freezing medium with a swab plus uninfected cells were compared to the mean concentration measured in plasma. Values from the swab preparations were all within threefold of the quantity measured in plasma (D. DeVange Panteleeff, S. Emery, B. A. Richardson, S. Bodrug, J. K. Kreiss and J. Overbaugh, unpublished data).

In processing and testing samples from study patients, vials containing swabs in freezing medium were vortexed to suspend secretions. Duplicate 200 µl aliquots were analyzed, and the means of the two assays were calculated. If the duplicates differed by more than threefold, the assays were repeated (two out of 72 samples). The lower limit of quantitation was defined as 125 copies of HIV-1 RNA/swab. Log10-transformed data were used for analyses of cervical HIV-1 RNA levels.

Cervical dry swab samples were prepared and tested for the presence of HIV-1 proviral DNA using nested PCR amplification of the gag gene, as described previously [8,13]. A minor modification was made in the concentration of MgCl2(2.5 mM), and bovine serum albumin was added to a final concentration of 0.01% to reduce inhibition by contaminants from the swab or blood cells. The assay is able to detect a single copy of HIV-1 DNA [13].

**Data analysis**

Data analysis was performed using S-plus (MathSoft Inc., Seattle, Washington, USA) and SPSS 9.0 (SPSS Inc., Chicago, Illinois, USA) software. Generalized estimating equations and Wilcoxon rank sum tests were used for comparisons of paired observations. For comparison of mean HIV-1 RNA levels in the presence or absence of infected cells, a linear mixed effects model was used to control for the correlation between samples from before and after treatment in individual patients. The method of restricted maximum likelihood was used for estimation of the variance components.

**Results**

A total of 1754 patients were screened of whom 683 (38.9%) were HIV-1 seropositive. Among the seroposi-
tive patients, 27 (4.0%) had \emph{N. gonorrhoeae}, 17 (2.5%) had \emph{C. trachomatis}, and 26 (3.8%) had non-specific cervicitis. Of the 70 patients with one of these conditions, 36 (51.4%) had a follow-up visit after cure (16 with \emph{N. gonorrhoeae}, seven with \emph{C. trachomatis}, and 13 with non-specific cervicitis). In 33 of the 36 patients, cervical infection or inflammation had resolved at the first follow-up visit. Two patients with \emph{N. gonorrhoeae} and one with \emph{C. trachomatis} required a second course of treatment, and their infections subsequently resolved. Characteristics of the 36 patients are shown in Table 1. Only 11.1% of the patients had CD4 cell counts < 200 × 10^6/l. The median time between the baseline visit and follow-up was 24.5 days (range 11–111 days; interquartile range, 17–33 days).

**Changes in cervical HIV-1 RNA levels and detection of infected cells with treatment**

Among the 36 patients with cervicitis, 26 (72.2%) demonstrated a decrease in cervical HIV-1 RNA after cure of the infection. Overall, the median HIV-1 RNA level in cervical secretions decreased from 4.05 to 3.24 log_{10} copies/swab after successful treatment \((P = 0.001)\) (Table 2). There were significant reductions in the quantity of cervical HIV-1 RNA among the subgroups with \emph{N. gonorrhoeae} and \emph{C. trachomatis}. For the 16 patients with \emph{N. gonorrhoeae}, the median cervical HIV-1 RNA level decreased from 3.94 to 3.28 log_{10} copies/swab \((P = 0.02)\). For the seven patients with \emph{C. trachomatis}, there was a reduction from 4.21 to 3.19 log_{10} copies/swab \((P = 0.02)\). Among patients with non-specific cervicitis \((n = 13)\), the change in cervical HIV-1 RNA concentration was not significant (3.67 to 3.54 log_{10} copies/swab; \(P = 0.66\)).

The assay used to detect HIV-1 infected cells was qualitative rather than quantitative; it measures the presence or absence of HIV-1 proviral DNA in cervical secretions. Infected cells were present in 24 (66.7%) of the 36 samples prior to treatment and in 15 (41.7%) after resolution of cervicitis \(\text{odds ratio (OR), 2.8; 95\% confidence interval (CI), 1.3–6.0; } P = 0.009\); Table 3). Among those with non-specific cervicitis, nine (69.2%) out of 13 had infected cells prior to treatment, compared with three (23.1%) following resolution \(\text{OR, 7.5; 95\% CI, 1.9–30.2; } P = 0.005\). The decreases in detection of HIV-1 infected cells were non-significant for the subgroups with \emph{N. gonorrhoeae} and \emph{C. trachomatis}.

Detection of HIV-1 infected cells was associated with higher mean HIV-1 RNA levels in cervical secretions (4.04 versus 2.99 log_{10} copies/swab; \(P < 0.0001\)).

**Cervical inflammation**

Treatment significantly reduced cervical inflammation. Overall, the median cervical PMNL count before treatment was 23.2, compared with 4.0 after treatment \((P < 0.001)\). The decrease in cervical PMNLs was significant among the subgroups with \emph{N. gonorrhoeae} \((15.7 \text{ to } 3.5; \text{OR, 0.004})\) and non-specific cervicitis \((33.3 \text{ to } 4.0; \text{OR, 0.001})\). Among patients with \emph{C. trachomatis}, the median cervical PMNL count decreased from 9.0 to 4.7 \((P = 0.18)\). Only three patients with \emph{N. gonorrhoeae} and none with \emph{C. trachomatis} had cervical PMNL counts greater than 30 at baseline.

**Discussion**

In this prospective study, it was found that effective treatment of cervicitis resulted in a 0.8 log_{10} (greater than sixfold) reduction in the quantity of HIV-1 RNA in cervical secretions. There was also an almost three-
fold reduction in the odds of detecting HIV-1 infected cells. Analysis of patients before and after treatment provides evidence for a causal association between cervicitis and shedding of HIV-1. This is the first study to show a decrease in the quantity of cervical HIV-1 RNA among patients treated for *N. gonorrhoeae* and *C. trachomatis*.

Cervicitis may increase shedding of HIV-1 through several mechanisms. These include increased viral replication in the presence of infection or inflammation, disruption of normal mucosa, and increased numbers of infected cells present in cervical secretions. HIV-1 replication may be increased by chlamydial infection in an *in vitro* model [18]. Furthermore, detection of cervical HIV-1 DNA is increased in the presence of elevated cytokines, suggesting that local inflammation may be an important factor influencing the viral concentration in cervical secretions [19].

The implications of these findings for the spread of HIV-1 depends on the relationship between detection of HIV-1 in body fluids and transmission of the virus. Detection of HIV-1 in maternal genital tract secretions is associated with mother-to-child transmission [20,21]. The presence of HIV-1 in genital secretions may also be an indicator for the risk of sexual transmission [22]. Increased HIV-1 in genital secretions of patients with STDs may provide a link in the biological pathway explaining the increased infectivity of these individuals [23].

It is unknown whether infected cells or cell free virus is more important in transmission of HIV-1 [24]. A strength of this study is that both HIV-1 RNA and DNA were assayed, demonstrating a strong correlation between the presence of infected cells and the total HIV-1 RNA level in cervical secretions. The mean HIV-1 RNA level was 11-fold higher in samples in which infected cells were detected.

The subgroup analyses raise questions about the relationship between infected cells and total HIV-1 RNA among patients with different causes of cervicitis. Among patients with *N. gonorrhoeae* and *C. trachomatis*, treatment significantly decreased the quantity of HIV-1 RNA whereas the decreases in detection of infected cells were non-significant, suggesting that viral gene expression in the cervical mucosa was increased in the presence of these STDs. In contrast, treatment of non-specific cervicitis reduced the prevalence of infected cells significantly whereas the decrease in the HIV-1 RNA level was non-significant. Based on estimates of HIV-1 turnover [25,26], a decrease in the prevalence of infected cells might be expected to lead to reduced viral RNA levels within 1–2 days. One possible explanation for these findings is that virus turnover in cervical secretions is slower than predicted by these estimates. Further work is needed to examine the effects of treatment for different causes of cervicitis using a quantitative rather than qualitative assay for HIV-1 infected cells.

We acknowledge the limitations of this study. The small number of patients in the subgroups makes it difficult to draw firm conclusions from comparisons between them. In addition, the study was designed to demonstrate the changes in HIV-1 shedding specifically after cure of cervicitis. The effect of treatment among women who were not cured was not investigated, and no control group of women without cervicitis was included. However, a strength of our prospective interventional design is that each woman with cervicitis served as her own control after resolution of the infection. Another limitation of this study is the sensitivity of the EIA used for *C. trachomatis*—only 92% for cervical specimens. Thus, a small number of patients with chlamydial cervicitis could have been classified as having non-specific cervicitis. Finally, the presence of blood on cervical swabs may influence the quantity of virus detected. However, the increased cervical friability seen with cervicitis may be one of the causal mechanisms for increased infectivity. By using the same technique to collect specimens before and after resolution of cervicitis, our results reflect the change in virus exposure from a surface to surface mucosal contact. Thus, despite its limitations, we feel that this study provides an accurate measure of the change in HIV-1 shedding with successful treatment of cervicitis.

In conclusion, we have demonstrated a significant decrease in shedding of HIV-1 after treatment of cervicitis. The relative decrease in genital HIV-1 RNA

<table>
<thead>
<tr>
<th>Table 3. Prevalence of HIV-1 infected cells before and after successful treatment of cervicitis.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before treatment</strong></td>
</tr>
<tr>
<td>Combined group (n = 36)</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em> (n = 16)</td>
</tr>
<tr>
<td><em>Chlamydia trachomatis</em> (n = 7)</td>
</tr>
<tr>
<td>Non-specific cervicitis (n = 13)</td>
</tr>
</tbody>
</table>

OR, Odds ratio; CI, confidence interval.
after treatment was comparable to that observed in a recent study after initiation of highly active antiretroviral therapy in antiretroviral naive patients [27]. Treatment of STDs is comparatively inexpensive and widely available, and may be a particularly important and cost-effective means of decreasing HIV-1 transmission in resource-poor settings where STDs are common.

Acknowledgements
The authors thank the nurses M. Wamagunda and F. Murigi for their dedication and hard work in the clinic. Thanks are due also to B. Chohan, A. Abdalla, G. Maina, K. Mushahame and S. Emery for excellent laboratory support. We thank Coast Provincial General Hospital for permission to use their clinical facilities. Finally, we thank the women who participated in this study, without whose time and effort the study would not have been possible.

Sponsorship: Supported by National Institutes of Health grants R01-AI39996 (J. K. K.) and AI38518 (J. O.). R. McClelland and C. Wang were scholars in the International AIDS Research and Training Program supported by the Fogarty International Center (D43-TW00007, T22-TW00001).

References