MAJOR ARTICLE

Contribution of HIV-1 Infection to Acquisition of Sexually Transmitted Disease: A 10-Year Prospective Study

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Background. Sexually transmitted diseases (STDs) enhance human immunodeficiency virus (HIV)–1 susceptibility, but few studies have examined the reciprocal effect of HIV-1 on STD acquisition.

Methods. Data from a prospective cohort study conducted among female sex workers in Mombasa, Kenya between 1993 and 2003 were used to determine the effect of HIV-1 infection on STD susceptibility. The cohort included 1215 HIV-1–seronegative women who underwent monthly HIV-1 and STD screening, of whom 238 experienced seroconversion to HIV-1 during follow-up. Andersen-Gill proportional-hazards models were used to compare the incidence rates for genital-tract infections (syphilis, genital ulcer disease [GUD], *Neisseria gonorrhoeae* infection, *Chlamydia trachomatis* infection, *Trichomonas vaginalis* infection, vulvovaginal candidiasis, and bacterial vaginosis) in HIV-1–seropositive versus HIV-1–seronegative women, after controlling for sexual behavior and other potential confounding factors.

Results. HIV-1 infection was associated with a significantly higher incidence of GUD (hazard ratio [HR], 2.8; 95% confidence interval [CI], 2.0–3.9), gonorrhea (HR, 1.6; 95% CI, 1.1–2.2), and vulvovaginal candidiasis (HR, 1.5; 95% CI, 1.3–1.8). The risks of GUD and vulvovaginal candidiasis increased with progressive levels of immunosuppression.

Conclusions. The increased incidence of genital-tract infections among HIV-1–seropositive women could promote the spread of both HIV-1 and other STDs, particularly in areas where these conditions are highly prevalent.

A bidirectional interaction between HIV-1 and the "classic" sexually transmitted diseases (STDs), referred to as "epidemiological synergy," has been proposed as one explanation for the rapid spread of the HIV-1 epidemic in some areas [1]. A recent systematic review identified 30 longitudinal studies that examined the effect of STDs on HIV-1 acquisition [2]. Several STDs and other genital-tract infections, including genital ulcer disease (GUD), nonulcerative STDs (e.g., *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection), and vaginal conditions such as vulvovaginal candidiasis and bacterial vaginosis, were found to significantly increase HIV-1 susceptibility. In contrast, the authors noted that few longitudinal studies have examined the reciprocal effect of HIV-1 on susceptibility to STDs and other genital-tract conditions. An increase in the risk of acquisition of these infections could have a substantial impact on global HIV-1 transmission, since genital-tract infections likely increase the infectiousness of HIV-1–seropositive individuals [3].

Understanding the interactions between HIV-1 and other STDs requires large prospective studies with frequent STD sampling and rigorous measurement of sexual behavior and other possible confounding factors [2]. The objective of the present investigation, which utilized data from a 10-year prospective cohort study of female sex workers (FSWs) in Kenya, was to deter-

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mine the effect of HIV-1 on the incidence of STDs and other genital-tract infections.

SUBJECTS AND METHODS

Participants and procedures. In 1993, a prospective open cohort study was initiated among FSWs attending a municipal clinic in Mombasa, Kenya. The primary aims of this study have been to identify risk factors for HIV-1 and STD acquisition and to describe the natural history of HIV-1 in this population. Informed consent was obtained from all participants. The study was approved by the institutional review boards of the University of Washington and the University of Nairobi, and the human experimentation guidelines of these institutions were followed. Study procedures have been detailed elsewhere [4]. Briefly, consenting women were screened for HIV-1, and those who were HIV-1 seronegative were invited to enroll in the cohort. At enrollment, a standardized interview regarding medical, gynecologic, and sexual history was administered. Focused interviews at monthly follow-up visits included collection of information on sexual behavior, contraceptive use, and symptoms during the past month.

At each visit, a physical examination, including a pelvic speculum examination, was performed. Standard criteria were used to identify STD syndromes. GUD was defined by the presence of an epithelial disruption of the cervix, vagina, or vulva. The presence of yellow or greenish cervical discharge was defined as mucopus. Vaginal discharge other than normal physiologic secretions was defined as abnormal.

Vaginal and cervical samples were collected for laboratory diagnosis of genital-tract infections. Blood samples were collected for HIV-1 and syphilis screening. Women who experienced seroconversion to HIV-1 were invited to continue with the same monthly follow-up interviews and examinations as HIV-1–seronegative women. Beginning in April 1998, CD4 cell counts were determined every 3 months for HIV-1–seropositive women.

Syndromic treatment was given to patients with signs or symptoms of genital-tract infections at the examination visit, according to Kenyan Ministry of Health guidelines. Women were asked to return for results 1 week after examination, and additional treatment was given, as needed, on the basis of the results of laboratory testing. Asymptomatic women who had a laboratory diagnosis of vaginal yeast colonization or bacterial vaginosis were not routinely treated. Individual HIV-1 risk-reduction counseling and free condoms were provided at each visit. For subjects who had experienced HIV-1 seroconversion, counseling included provision of information on STD risk and on the risk of HIV-1 transmission to sex partners.

Laboratory methods. Screening for HIV-1 was performed using ELISA (Detect-HIV; Biochem Immunosystems). Positive results were confirmed using a second ELISA (Recombigen; Cambridge Biotech). Screening for syphilis was performed every 3 months using rapid plasma reagin (RPR) (Becton Dickinson). Reactive samples were confirmed using a *Treponema pallidum* hemagglutination assay (TPHA) (Biotec). Incident syphilis was defined as a 4-fold increase in RPR titer with a positive TPHA result. Culture of endocervical secretions for *N. gonorrhoeae* was performed on modified Thayer-Martin media. ELISA (Microtrak; Syva) was performed for diagnosis of *C. trachomatis*. Use of this assay was discontinued in April 1999, because of a low incidence of chlamydia in the cohort. The presence of yeast and *Trichomonas vaginalis* was detected by light microscopy of a saline wet preparation. Bacterial vaginosis was evaluated by microscopy of a vaginal Gram stain [5]. Beginning in April 1998, CD4 cell counts were determined using either a manual (Cytosphere; Coulter) or a semiautomated (Zymmune; Bartels) method.

Statistical analysis. Data analysis was performed using SPSS (version 10; SPSS) and S-Plus 2000 (MathSoft). All HIV-1–seronegative women who enrolled in the cohort and had at least 1 follow-up visit were considered for inclusion in this analysis. Visits at which women were pregnant were excluded, as were visits that took place as part of 2 studies of the vaginal microbicide nonoxynol-9 [6, 7].

Andersen-Gill proportional-hazard models were used to compare the risk of genital-tract infections in HIV-1–seropositive versus HIV-1–seronegative women. All HIV-1–seropositive women in this analysis experienced seroconversion during follow-up, and HIV-1 serostatus was included as a time-dependent variable. Because HIV-1 infection is associated with many other factors that increase STD risk, analyses controlled for several potential confounding variables. These included the following baseline characteristics: education ($\leq$8 vs. >8 years), parity ($\leq$2 vs. >2 children), workplace (bar vs. nightclub, since bar work has been associated with increased HIV-1 risk in this population [4]), and douching practices (with soap vs. no douching or douching with water only). Education and parity were dichotomized at the median. Models were also adjusted for the following time-dependent covariates: age (<25, 25–29, 30–34, 35–39, and $\geq$40 years), duration of prostitution ($\leq$1, 2–4, 5–9, and $\geq$10 years), number of sex partners ($\leq$1 vs. >1 partner/week), frequency of sexual activity ($\leq$2 vs. >2 times/week), condom use (<100% vs. 100%), contraceptive use (oral contraceptive pills vs. depot medroxyprogesterone acetate vs. none or tubal ligation), calendar year (to account for secular changes in STD risk in this community [8]), and time since last clinic visit (dichotomized at the median for the cohort: $\leq$35 days vs. >35 days).

Because the effect of hormonal contraception may persist after discontinuation or a change in contraceptive methods, women were considered to be exposed to a hormonal-contraception method for 85 days after the last reported use, as in our previous analyses [4, 9]. The comparison group for hormonal contraceptives was women reporting either no contra-

ceptive method or tubal ligation. Because many women, including those using hormonal methods of contraception, used condoms for STD protection, condom use was included as a separate variable. Visits at which use of an intrauterine device or Norplant were reported were excluded because of small numbers. For the sexual-behavior variables (number of sex partners, frequency of sexual activity, and percent condom use), an average was calculated for each year of follow-up, to capture average behavior over time [8]. The dichotomous categories for these sexual-behavior variables were defined by the median for the cohort, as in our previous analyses [9].

To evaluate the effect of progressive immunosuppression on the risk of acquiring genital-tract infections, additional models were constructed to compare STD risk among HIV-1–seropositive women with CD4 cell counts ⩾500, 200–499, and <200 cells/μL and HIV-1–seronegative women. For visits at which the CD4 cell count was not measured, the count at that visit was defined as the most recent preceding value.

Table 1. Characteristics of subjects at enrollment (n = 1215).

Characteristic	Value
Demographic	
Age, years	26 (22–31)
Education, years	8 (6–10)
Duration of prostitution, years	1 (0.2–3.1)
Works in bar (vs. nightclub),[a] no. (%) of subjects	841 (75)
Behavioral	
Frequency of sexual activity, times per week	2 (1–3)
No. of sex partners per week	1 (1–2)
100% condom use,[b] no. (%) of subjects	736 (62)
Douches with soap, no. (%) of subjects	867 (71)
Obstetrical/gynecological	
Parity, no. of children	2 (1–3)
Hormonal contraceptive use, no. (%) of subjects	
Depot medroxyprogesterone acetate	246 (20)
Oral contraceptive pills	175 (14)

NOTE. Data are median (interquartile range), unless otherwise indicated.
[a] For the women who worked either at a bar or at a nightclub, n = 1124.
[b] For the women who reported a frequency of sexual activity of >0 times/week, n = 1191.

RESULTS

Characteristics of the study population. Between February 1993 and February 2003, 1498 women were enrolled in the cohort. Of these, 1215 (81%) met the inclusion criteria for the present analysis. The median duration of follow-up was 617 days (interquartile range [IQR], 162–1779 days), and the median time between follow-up visits was 35 days (IQR, 28–63 days). A total of 3140 person-years of follow-up were accrued. Two hundred thirty-eight women experienced seroconversion to HIV-1 (incidence, 8.8/100 person-years). Baseline characteristics of the study population are presented in table 1.

Risk of genital-tract infections by HIV-1 serostatus. There was a high incidence of STDs and other genital-tract conditions during follow-up (table 2). In multivariate analyses adjusted for sexual behavior and other potential confounding factors, women who were HIV-1 seropositive had significantly higher rates of GUD (hazard ratio [HR], 2.8; 95% confidence interval [CI], 2.0–3.9), gonorrhea (HR, 1.6; 95% CI, 1.1–2.2), and vulvovaginal candidiasis (HR, 1.5; 95% CI, 1.3–1.8), compared with HIV-1–seronegative women. There was also a trend for more frequent detection of trichomoniasis among HIV-1–seropositive versus HIV-1–seronegative women (HR, 1.3; 95% CI, 1.0–1.7). The rates of syphilis, chlamydia, cervical mucopus, cervicitis, bacterial vaginosis, and abnormal vaginal discharge did not differ significantly by HIV-1 serostatus.

Risk of genital-tract infections by CD4 cell count. For outcomes for which HIV-1 seropositivity was associated with an overall increased risk (GUD, *N. gonorrhoeae* infection, vulvovaginal candidiasis, and *T. vaginalis* infection), we further evaluated the risk of the genital-tract infection in relation to progressive levels of immunosuppression. HIV-1–seropositive women were stratified by CD4 cell count and compared with seronegative women. The risk of GUD in HIV-1–seropositive women with decreasing CD4 cell counts increased in a stepwise fashion, compared with the risk in seronegative women, with an HR of 2.5 (95% CI, 1.4–4.6) among women with ⩾500 cells/μL, an HR of 3.7 (95% CI, 2.3–6.0) among women with 200–499 cells/μL, and an HR of 5.0 (95% CI, 1.5–16.8) among women with <200 cells/μL. Likewise, the risk of vulvovaginal candidiasis in HIV-1–seropositive women with decreasing CD4 cell counts showed stepwise increases compared with the risk in seronegative women, with an HR of 0.9 (95% CI, 0.6–1.5) among women with ⩾500 cells/μL, an HR of 1.6 (95% CI, 1.2–2.0) among women with 200–499 cells/μL, and an HR of 2.1 (95% CI, 1.1–4.0) among women with <200 cells/μL. Recent analyses have differentiated *Candida* vaginitis from asymptomatic *Candida* colonization [10], and we repeated our analysis with the definition of vulvovaginal candidiasis restricted to women who reported vaginal itching or discharge when asked about these symptoms. When this definition was used, the relationship between immunosuppression and vulvovaginal candidiasis was even more striking for those with CD4 cell counts <200 (HR, 5.4; 95% CI, 1.7–17.9; P = .005). However, there was no increased risk, compared with that for seronegative women, for HIV-1–seropositive women with CD4 cell counts of 200–499 cells/μL (HR, 1.1; 95% CI, 0.6–2.1; P = .7) or ⩾500 cells/μL (HR, 1.0; 95% CI, 0.5–2.1; P = 1.0). Among HIV-1–seropositive women, progressive decreases in CD4 cell count were not associated with increased risk of gonorrhea or trichomoniasis, compared with seronegative women.

Condom use and risk of STD acquisition. Overall, the incidence rates for gonorrhea (HR, 0.6; 95% CI, 0.4–0.8; P< .001), chlamydia (HR, 0.6; 95% CI, 0.4–0.9; P = .01), cervical

Table 2. Incidence of sexually transmitted diseases and other genital-tract conditions and multivariate association with HIV-1 seropositivity.

Condition	Incidence per 100 person-years (no. of cases)	HR (95% CI)[a]	*P*
Syphilis	2.3 (73)	1.2 (0.6–2.5)	.7
Genital ulcer disease	7.3 (229)	2.8 (2.0–3.9)	<.001
Neisseria gonorrhoeae infection	13.3 (418)	1.6 (1.1–2.2)	.006
Chlamydia trachomatis infection[b]	9.0 (146)	1.2 (0.7–2.2)	.4
Cervicitis	43.3 (1360)	0.9 (0.7–1.1)	.2
Cervical mucopus	20.4 (642)	1.1 (0.8–1.6)	.6
Bacterial vaginosis	169.5 (5323)	1.1 (0.9–1.3)	.3
Vulvovaginal candidiasis	55.6 (1745)	1.5 (1.3–1.8)	<.001
Trichomonas vaginalis infection	25.9 (813)	1.3 (1.0–1.7)	.08
Abnormal vaginal discharge	75.7 (2377)	1.1 (0.9–1.3)	.5

NOTE. Andersen-Gill proportional-hazard models were used to generate a hazard ratio (HR) for HIV-1–seropositive women compared with women who were HIV-1 seronegative. Models were adjusted for the following baseline variables: education (≤8 vs. >8 years), parity (≤2 vs. >2 children), workplace (bar vs. nightclub), and douching practices (with soap vs. no douching or douching with water only). Education and parity were dichotomized at the median. Models were also adjusted for the following time-dependent covariates: age (<25, 25–29, 30–34, 35–39, and ≥40 years), duration of prostitution (≤1, 2–4, 5–9, and ≥10 years), no. of sex partners (≤1 vs. >1 partner/week), frequency of sexual activity (≤2 vs. >2 times/week), condom use (<100% vs. 100%), oral contraceptive pill use, injectable contraceptive use, calendar year, and time since last clinic visit (dichotomized at the median for the cohort: ≤35 days vs. >35 days). HIV-1 serostatus was analyzed as a time-dependent variable. CI, confidence interval.

[a] For HIV-1–seropositive vs. HIV-1–seronegative subjects.

[b] Total person-years of follow-up was 1614.

mucopus (HR, 0.8; 95% CI, 0.6–1.0; $P = .03$), GUD (HR, 0.7; 95% CI, 0.5–1.0; $P = .04$), and bacterial vaginosis (HR, 0.9; 95% CI, 0.8–1.0; $P = .005$) were significantly lower among women who reported 100% condom use, compared with women who reported less frequent use of condoms. There was also a trend for a lower incidence of cervicitis (HR, 0.9; 95% CI, 0.8–1.0; $P = .06$). The incidence of trichomoniasis, vulvovaginal candidiasis, syphilis, and vaginal discharge did not vary significantly in relation to reported condom use.

DISCUSSION

In this large prospective cohort study of FSWs in Mombasa, Kenya, HIV-1 seropositivity was associated with significantly higher incidence of GUD, gonorrhea, and vulvovaginal candidiasis, after rigorously controlling for differences in sexual behavior and other potential confounding factors. A trend for increased incidence of trichomoniasis in HIV-1–seropositive women, compared with that in seronegative women, was also observed. A strong association between genital-tract infections and increased HIV-1 susceptibility has previously been demonstrated in several populations, including this cohort [2, 4]. STDs also increase the infectiousness of HIV-1–seropositive individuals [11–13]. The results presented here provide evidence of an additional mechanism for epidemiological synergy between HIV-1 and other STDs. In addition, an increased risk for STDs could have an effect on HIV-1 pathogenesis by increasing plasma HIV-1 load [14], which could lead to accelerated disease progression.

Our findings from this 10-year prospective study agree with the results of other investigations, which have suggested that HIV-1 may increase the incidence of GUD, herpes simplex virus type 2 (HSV-2), chancroid, gonorrhea, and vaginal candidiasis [10, 15–19]. There are several plausible explanations for these findings. HIV-1 may influence the susceptibility to genital-tract infections or the duration, response to treatment, recurrence rate, or severity of genital-tract infections. For example, women who are HIV-1 seropositive are more likely to experience reinfection with the same strain of *N. gonorrhoeae,* possibly because of a defect in strain-specific immunity [16]. HIV-1–mediated CD4 cell depletion promotes HSV reactivation, which could explain an increase in the incidence of GUD [20]. Finally, in HIV-1–seropositive women, an increased incidence of mucosal candidiasis may be related to multiple factors, including immunosuppression and direct effects from higher levels of HIV-1 viremia [10]. In support of this hypothesis, we found that the risk of GUD and vulvovaginal candidiasis increased in a stepwise fashion with progressive CD4 cell depletion. In contrast, the risk of gonorrhea and trichomoniasis did not follow a clear pattern in relation to CD4 cell count. However, unlike GUD and vulvovaginal candidiasis, new episodes of gonorrhea and trichomoniasis require reexposure.

Several features of this study support the validity of our

findings. First, the prospective cohort design with frequent screening for genital-tract infections allowed accurate determination of the timing of the exposure (HIV-1 infection) in relation to the outcome (genital-tract infection). In contrast to cross-sectional studies, this analysis helps to establish a clear direction of causality. HIV-1 infection increases the risk of subsequent STDs and other genital-tract infections. Second, the study included rigorous control for reported sexual behavior and other potential confounding factors. Such carefully adjusted analyses are essential for the study of the relationship between HIV-1 and other STDs, since both share a common transmission route. When proper care is taken, behavioral self-reports can provide valid data [21]. Nevertheless, the possibility that underreporting of risky behavior could have decreased the ability of adjusted analyses to control for differences in risk between HIV-1–seropositive and HIV-1–seronegative women must be considered. In this context, it was reassuring that not all STDs were increased among HIV-1–seropositive versus HIV-1–seronegative women, suggesting that the associations we observed were not simply a result of uncontrolled confounding by higher-risk behavior among HIV-1–seropositive women. Third, participants were asked to return for monthly examinations, allowing detection of asymptomatic as well as symptomatic infections and minimizing bias due to patients attending the clinic only when symptomatic. Finally, the large size of the cohort, long duration of follow-up, and high incidence of genital-tract infections enhanced both the precision of our risk estimates and the study power.

There are limitations to this study. GUD is a syndrome that encompasses a heterogeneous group of etiologies. Most cases of GUD observed in the cohort were clinically consistent with reactivation of genital herpes, and the seroprevalence of HSV-2 in this population is very high [22]. This study was not designed to distinguish the independent effects of HIV-1 on the various infectious and noninfectious causes of GUD. However, from the perspective of our study, the association between HIV-1 and increased GUD is important regardless of the etiology, since syndromic GUD has been associated with heightened HIV-1 susceptibility and infectiousness. An additional limitation is the fact that detection of *N. gonorrhoeae* by culture and of *C. trachomatis* by ELISA are less sensitive than detection by nucleic acid amplification techniques [23]. The limited sensitivity of these assays would be unlikely to introduce bias as long as missed cases occurred with the same frequency among HIV-1–seropositive and HIV-1–seronegative women. However, this study cannot rule out the possibility that HIV-1 infection influenced the number of organisms present, which might, in turn, influence the sensitivity of the assays. Finally, it should be noted that sex partners and networks were not evaluated in this study, although these factors also influence the risk of acquiring STDs.

Although many studies have shown that STDs and other genital-tract infections increase the risk of HIV-1 acquisition, few have measured the effect of HIV-1 on the risk of STD acquisition [2]. The present study provides a detailed quantitative analysis of this component of the bidirectional interaction between HIV-1 and other genital-tract infections. The findings also demonstrate that women reporting 100% condom use had a significantly lower risk of several STDs, highlighting the importance of condoms as an intervention strategy for reducing the risk of genital-tract infections. The high incidence of GUD and cervical and vaginal infections associated with HIV-1 indicates the need for more intensive treatment and prevention of these conditions as a means of decreasing HIV-1 infectivity [12, 13, 24]. As antiretroviral therapy becomes more widely available, it will also be important to determine whether recovery of the immune system reduces the risk of genital-tract infections and whether suppression of plasma viremia limits genital HIV-1 shedding associated with STDs.

In conclusion, programs directed at HIV-1–seropositive individuals with STDs may have a substantial impact on the spread of HIV-1. Operational research is needed to determine the ideal frequency of screening and appropriate treatment regimens for specific populations. The findings presented here highlight the need for both research and program support to implement targeted screening and treatment for genital-tract infections in HIV-1–seropositive individuals, as an HIV-1 prevention strategy.

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