MAJOR ARTICLE

Recent Herpes Simplex Virus Type 2 Infection and the Risk of Human Immunodeficiency Virus Type 1 Acquisition in India

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To estimate the impact of prevalent and incident herpes simplex virus type 2 (HSV-2) infection on the acquisition of human immunodeficiency virus type 1 (HIV-1), stored serum samples from a cohort of 2732 HIV-1–seronegative patients attending 3 sexually transmitted infection clinics and 1 reproductive tract infection clinic in Pune, India, were screened for HSV-2–specific antibodies. Incident HSV-2 infection was defined serologically as "recent" if a negative result of testing for HSV-2 could be documented within the previous 6 months or "remote" if >6 months had elapsed since the last negative test result. The prevalence of HSV-2 at enrollment was 43%. The HSV-2 incidence was 11.4 cases/100 person-years, and the HIV-1 incidence was 5.8 cases/100 person-years. The adjusted hazard ratios of HIV-1 acquisition from exposure to HSV-2 infection were 1.67 for prevalent HSV-2, 1.92 for remote incident HSV-2, and 3.81 for recent incident HSV-2. Recent incident HSV-2 infection was associated with the highest risk of HIV-1 in this study, which suggests that prevention of HSV-2 infection may reduce the risk of HIV-1 acquisition.

Sexual transmission of human immunodeficiency virus (HIV) is facilitated by the presence of genital ulcer disease [1–6]. Herpes simplex virus type 2 (HSV-2) infection is the most common cause of genital ulcer disease in both developed and developing countries [7–12]. A clear relationship has been established between genital herpes and HIV acquisition, but almost all studies have evaluated prevalent genital herpes infection [13–17]. Recent studies conducted in Zimbabwe and Tanzania suggest that an association exists between incident HSV-2 infection and HIV-1 acquisition [18, 19]. HSV-2 and its potential interaction with HIV have emerged as a major public health problem for countries facing the global HIV-1 pandemic [20].

Our group found the presence of a clinically apparent genital ulcer at the time of screening in 79% of patients with sexually transmitted infections (STIs) who had acute HIV-1 infection in India [3]. This, coupled with the fact that HSV DNA was isolated from 26% of genital ulcer specimens in our clinic patients, led us to believe that genital herpes was a major factor in the current HIV-1 epidemic in India [8]. The high rates of both

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Informed consent was obtained from all patients participating in the study. The human experimentation guidelines of the US Department of Health and Human Services and of participating institutions were followed in conducting this research.

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HSV-2 and HIV-1 in our study population and a schedule of frequent follow-up visits afforded a unique opportunity to study the association of these 2 viral STIs with greater attention to the relative timing of these infections and, ultimately, to provide insight into any causal association that may exist between incident HSV-2 infection and HIV-1 acquisition.

Recent serologic data illustrate that the majority of incident cases of HSV-2 infection are clinically asymptomatic [21–23]. The commercial availability of type-specific serologic assays for HSV, coupled with our clinical data on genital ulcer disease, provided the additional opportunity to explore what role, if any, asymptomatic HSV-2 infection plays in HIV-1 acquisition.

PATIENTS, MATERIALS, AND METHODS

Study participants. HIV-1–seronegative patients attending 3 referral STI clinics and a reproductive tract infection clinic were enrolled in a prospective study of HIV-1 infection in Pune, India, from May 1993 through April 2000. The study population represents a mixture of male patients with STIs, female partners of male patients with STIs, female commercial sex workers, and women with reproductive tract infections.

Study design. The original study design has been described elsewhere [2]. In brief, all patients received pre- and posttest counseling for HIV and other STIs. At screening, participants were interviewed using a structured questionnaire about demographic characteristics, STI history, medical history, sexual behavior, HIV/AIDS knowledge, and clinical symptoms. At the time of the physical examination and interview, investigators and participants were unaware of participants' HIV antibody status. The presence of a genital ulcer was assessed at each visit by physical examination. Blood was obtained for serologic testing for HIV at enrollment and follow-up visits. Unused serum was stored at −20°C for future testing. All participants were informed of the confidentiality of their test results. The present analysis was a retrospective cohort study using stored serum.

Serologic testing for HIV. Serum samples were screened with a commercially available EIA kit for identification of HIV-1 and HIV-2 antibodies (Recombigen HIV-1/HIV-2; Cambridge Biotech). Positive results of EIA were confirmed with a rapid test for HIV-1 and HIV-2 (Recombigen HIV-1/HIV-2 Rapid Test Device; Cambridge Biotech). When the results of these tests were discrepant, a third EIA or Western blot assay (Cambridge Biotech) was used. Western blot assays were interpreted according to the Centers for Disease Control and Prevention criteria [24].

Serologic testing for HSV-2. Serologic testing for HSV-2 was performed on all baseline specimens, using a type-specific, HSV-2 gpG2 IgG EIA (Focus Technologies). Incident HSV-2 infection was defined as infection in a patient who was seronegative for HSV-2 at the enrollment visit and seropositive at a follow-up visit. HSV-2 serostatus was not known to the participants at the time of the study.

Statistical analysis. The time of incident HSV-2 and HIV-1 infection was estimated, by interpolation, to be the midpoint between the last negative and the first subsequent positive test result. Risk factors for prevalent HSV-2 infection were assessed using χ^2 and Fisher's exact tests. Adjusted odds ratios (ORs) were estimated from a logistic regression model, with fit assessed using the Hosmer-Lemeshow goodness-of-fit test [25]. Crude HSV-2 and HIV-1 incidence rates were calculated as number of seroconversions divided by the summed person-years of follow-up. A Poisson distributed variable was used to calculated 95% confidence intervals (CIs) [26]. Unadjusted rate ratios were computed as the ratio of the incidence rate in the category of interest divided by the rate in the referent category, with 95% CIs based on exact Poisson methods.

Cox proportional hazards regression models with both time-independent and time-dependent covariates were used for multivariate analyses of incident infection. Participants who seroconverted were matched to the risk set by follow-up time: data collected at the visit at which seroconversion was demonstrated were compared with data collected from the risk set at the visit matching closest in terms of follow-up time. Variables for the multivariate analyses were chosen on the basis of previously identified risk factors for HIV-1 infection in this cohort, and the results of forward stepwise regression [2]. Participants with positive results of serologic testing for HSV-2 at enrollment were considered to have prevalent HSV-2 infection. Because the main hypothesis was that recent exposure to incident HSV-2 infection was independently associated with HIV-1 acquisition, incident HSV-2 cases were classified as either remote or recent. Remote incident infections were defined as those acquired after study entry but with >6 months since the last negative HSV-2 test, and recent incident infections as those where a negative HSV-2 test was documented within the past 6 months. Follow-up time for all participants who seroconverted to HSV-2 positive was classified according to these time-dependent criteria and used in the calculation of HIV-1 incidence rates, with the initial 6 months after HSV-2 seroconversion classified as recent exposure and any additional follow-up time classified as remote. Thus, a participant could potentially transition from the recent to the remote exposure category and contribute person-years to both. All statistical analyses were performed using SAS software (version 8.0; SAS Institute).

RESULTS

Characteristics of the study participants. Of 2732 persons enrolled, 2260 were male, 9 were hijra (eunuchs), and 463 were female; 1175 participants (43%) had HSV-2 antibodies. The demographic characteristics of study participants are described

in table 1. The median duration of follow-up was 10.7 months, and the median number of follow-up visits was 3. Of the enrolled participants who attended their first follow-up visit, 62.4% returned for the second visit. Participants with more lifetime sex partners (10–99 partners) tended to have a longer duration of follow-up (median, 13.1 months) but were less likely to report for the regular quarterly follow-up visit. Participants with high school–level education tended to have a longer duration of follow-up (median, 12.9 months) and were more likely to report for the regular quarterly follow-up visit. HSV-2 prevalence did not differ significantly by duration of follow-up, but it was significantly associated with an average interval between visits of >6 months (prevalence was 47.1% for an average interval of >6 months and 41.3% for an average interval of ≤6 months; $P = .005$).

Characteristics of study participants with prevalent HSV-2 infection. Of HSV-2–seropositive individuals, 44% reported no history of genital ulcer disease. Multiple associations between genital ulcer disease and prevalent HSV-2 infection were identified in the unadjusted analysis (table 1) and were included in the multivariate model. The prevalence of HSV-2 infection at baseline was higher among female patients with STIs than among male patients with STIs (50.9% vs. 38.1%; adjusted OR, 2.50; 95% CI, 1.70–3.68). Commercial sex work was the strongest independent risk factor identified (adjusted OR, 11.06; 95% CI, 5.91–20.7). Other independent risk factors associated with prevalent HSV-2 infection included age >30 years, marital status, greater lifetime number of sex partners, history or clinical presentation of genital ulcer, and earlier calendar period of screening (1993–1996) (table 1).

Incidence and predictors of HSV-2 infection. During the study, 217 participants seroconverted to HSV-2 positive, resulting in a crude HSV-2 incidence rate of 11.4 cases/100 person-years (95% CI, 9.9–13.0 cases/100 person-years). Risk factors for HSV-2 acquisition identified in the unadjusted analysis included earlier calendar period of follow-up (1993–1996), younger age, female sex work, lower education level, living away from family, lack of condom use, genital lesion at the current or a previous visit, and coincident HIV-1 infection (table 2). Independent predictors of incident HSV-2 infection included the presence of a genital lesion at a previous clinic visit only (adjusted hazard ratio [HR], 3.81; 95% CI, 2.77–5.23), the presence of a genital lesion at the current clinic visit (adjusted HR, 2.73; 95% CI, 1.56–4.79), and the presence of a genital lesion at both the current clinic visit and a previous visit (adjusted HR, 5.77; 95% CI, 3.91–8.52). When the group of male patients with STIs was used as a reference in the multivariate model, hijras and female sex workers had significantly higher HSV-2 incidence rates (adjusted HR, 5.87; 95% CI, 1.44–23.9; and adjusted HR, 3.90; 95% CI, 1.99–7.62, respectively).

HSV-2 infection and the risk of HIV-1 acquisition. The unadjusted rate ratio of HIV-1 acquisition among participants exposed to prevalent HSV-2 infection was 2.07 (95% CI, 1.53–2.83; table 3). Of the 224 participants with incident HIV-1 infection, 28 were found to have both incident HSV-2 infection and incident HIV-1 infection during the follow-up period (figure 1). For the majority of these 28 participants ($n = 22$), serologic evidence of these 2 infections was detected simultaneously. The unadjusted rate ratio of HIV-1 acquisition among participants exposed to remote incident HSV-2 infection was found to be 2.08 (95% CI, 1.20–3.48); among participants exposed to recent incident HSV-2 infection, the rate ratio was 6.26 (95% CI, 2.59–13.07). The adjusted HR of HIV-1 acquisition increased with relative timing of HSV-2 infection, from 1.67 (95% CI, 1.22–2.30) among those exposed to prevalent HSV-2 infection to 1.92 (95% CI, 1.15–3.21) among those exposed to remote incident HSV-2. Exposure to recent incident HSV-2 infection conferred a 3.81-fold (95% CI, 1.81–8.03) increased hazard of HIV-1 acquisition (figure 2).

Interaction between HSV-2 serostatus and genital ulcer disease. The interaction between clinically apparent or self-reported genital ulcer disease and HSV-2 serostatus was investigated in a proportional hazards model of HIV-1 acquisition. Of the 217 individuals with serologic evidence of incident HSV-2 infection, 51 (23.5%) had a genital lesion documented at the same visit at which seroconversion was demonstrated. A statistically significant interaction was found between prevalent HSV-2 infection and clinically apparent or self-reported genital ulcer (symptomatic infection) and the risk of HIV-1 infection. The presence of asymptomatic prevalent HSV-2 infection (no clinically apparent or self-reported genital ulcer) conferred an adjusted HR for HIV-1 infection of 2.14 (compared with no genital ulceration and negative results of serologic testing for HSV-2; 95% CI, 1.32–3.46). Symptomatic prevalent HSV-2 infection conferred an adjusted HR of 5.06 (95% CI, 3.19–8.03). No statistically significant interaction of clinically apparent or self-reported genital ulcer and incident HSV-2 infection with the risk of HIV-1 infection was found.

DISCUSSION

The findings of this study demonstrate that individuals who experience incident HSV-2 infections are at the greatest risk for HIV-1 acquisition, compared with individuals who are not infected with HSV-2 or who have prevalent HSV-2 infection. Individuals with serologic evidence of recent incident HSV-2 infection in our study had the highest HIV-1 incidence (adjusted HR, 3.55) when the analysis was controlled for other sexual risk behaviors, which illustrates that incident infection with this common sexually transmitted virus is independently associated with HIV-1 acquisition (figure 2).

The majority of new infections with HSV-2 have no clinical

Table 1. Characteristics of individuals participating in a study of human immunodeficiency virus type 1 incidence in Pune, India, and associations with herpes simplex virus type 2 (HSV-2) prevalence.

Characteristic	Total no. (%) of participants	HSV-2–seropositive participants				
		No. (%) of participants	Unadjusted analysis		Adjusted analysis	
			OR (95% CI)	*P*	OR (95% CI)[a]	*P*
All participants	2732 (100)	1175 (43.0)	—	—	—	—
Screening period, years						
1993–1996	1984 (72.6)	909 (45.8)	1.00 (referent)		1.00 (referent)	
1997–2000	748 (27.4)	266 (35.6)	0.65 (0.55–0.78)	.001	0.76 (0.61–0.95)	.017
Sex and risk group						
Male patient with STI	2260 (82.7)	861 (38.1)	1.00 (referent)		1.00 (referent)	
Hijra (eunuch)	9 (0.3)	5 (55.6)	2.03 (0.56–7.39)	.32	2.64 (0.50–13.9)	.25
Female patient with STI	271 (9.9)	138 (50.9)	1.69 (1.31–2.17)	.001	2.50 (1.70–3.68)	<.001
Female sex worker	192 (7.0)	171 (89.1)	13.23 (9.15–19.1)	.001	11.06 (5.91–20.7)	<.001
Age group, years						
<20	327 (12.0)	97 (29.7)	1.00 (referent)		1.00 (referent)	
20–24	894 (32.7)	322 (36.0)	1.34 (1.02–1.76)	.05	1.20 (0.89–1.63)	.23
25–29	619 (22.7)	263 (42.5)	1.75 (1.32–2.33)	.001	1.35 (0.97–1.87)	.08
⩾30	892 (32.7)	493 (55.3)	2.93 (2.23–3.84)	<.001	1.92 (1.37–2.70)	<.001
Marital status						
Never married	1347 (49.3)	452 (33.6)	1.00 (referent)		1.00 (referent)	
Married	1223 (44.8)	609 (49.8)	1.96 (1.67–2.30)	.001	1.40 (1.11–1.76)	.004
Separated[b]	162 (5.9)	114 (70.4)	4.70 (3.37–6.55)	.001	1.61 (1.02–2.56)	.04
Living away from spouse/family						
No	2059 (75.5)	854 (41.5)	1.00 (referent)		1.00 (referent)	
Yes	669 (24.5)	321 (48.0)	1.30 (1.09–1.55)	.003	1.06 (0.85–1.31)	.62
Level of education						
None	457 (16.8)	277 (60.6)	1.00 (referent)		1.00 (referent)	
Primary/middle school	1075 (39.4)	469 (43.6)	0.50 (0.40–0.63)	<.001	1.01 (0.77–0.33)	.92
High school	1196 (43.8)	428 (35.8)	0.36 (0.29–0.45)	<.001	0.88 (0.66–1.17)	.37
Lifetime no. of sex partners						
1	635 (23.2)	235 (37.0)	1.00 (referent)		1.00 (referent)	
2–9	1338 (49.0)	501 (37.4)	1.02 (0.84–1.24)	.85	1.02 (0.81–1.29)	.84
10–99	442 (16.2)	211 (47.7)	1.56 (1.22–1.99)	.001	1.42 (1.06–1.89)	.018
⩾100	175 (6.4)	141 (80.6)	7.06 (4.85–10.27)	.001	1.63 (0.96–2.78)	.07
Condom use						
No sex partners in past 3 months	1068 (39.1)	426 (39.9)	1.00 (referent)		1.00 (referent)	
Never	1094 (40.0)	471 (43.1)	1.14 (0.96–1.35)	.14	0.84 (0.68–1.04)	.11
Sometimes	324 (11.9)	166 (51.2)	1.58 (1.23–2.03)	.001	0.80 (0.59–1.10)	.16
Always	246 (9.0)	112 (45.5)	1.26 (0.95–1.67)	.11	0.89 (0.63–1.25)	.50
History of genital ulcer						
No	1537 (56.3)	514 (33.4)	1.00 (referent)		1.00 (referent)	
Yes	1187 (43.4)	656 (55.3)	2.46 (2.11–2.87)	.001	2.34 (1.96–2.79)	<.001
Genital ulcer on examination						
No	1776 (65.0)	675 (38.0)	1.00 (referent)		1.00 (referent)	
Yes	919 (33.6)	475 (51.7)	1.75 (1.49–2.05)	.001	2.03 (1.68–2.45)	<.001
Genital discharge on examination						
No	2131 (78.0)	879 (41.3)	1.00 (referent)		1.00 (referent)	
Yes	570 (20.9)	270 (47.4)	1.28 (1.07–1.54)	.01	0.82 (0.64–1.05)	.12
VDRL test results						
Negative	2397 (87.7)	1003 (41.8)	1.00 (referent)		1.00 (referent)	
Positive	280 (10.2)	144 (51.4)	1.47 (1.15–1.88)	.003	1.04 (0.78–1.38)	.80

NOTE. Data on whether participants were living away from spouse/family were missing for 4 participants; level of education, for 4; lifetime no. of sex partners, for 142; history of genital ulcer, for 8; presence of genital ulcer on examination at baseline visit, for 37; presence of genital discharge on examination at baseline visit, for 31; and baseline VDRL test results, for 55. CI, confidence interval; OR, odds ratio; STI, sexually transmitted infection.

[a] Adjusted for all other variables shown. Hosmer-Lemeshow goodness-of-fit statistic for the logistic regression model was 7.79, with 8 *df* ($P = .45$).

[b] Individuals who were separated from their spouse, divorced, or widowed.

Table 2. Risk factors for incident herpes simplex virus type 2 (HSV-2) infection among patients at 3 sexually transmitted infection (STI) clinics and 1 reproductive tract infection clinic in Pune, India, May 1993 through April 2000.

Characteristic	No. of participants who seroconverted to HSV-2 positive	Person-years	HSV-2 incidence, cases/100 person-years (95% CI)	Unadjusted analysis		Adjusted analysis	
				Rate ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
All participants	217	1896.6	11.4 (9.9–13.0)	—	—	—	—
Screening period, years							
1993–1996	164	1047.0	15.7 (13.4–18.3)	1.00 (referent)		—	—
1997–2000	53	849.6	6.2 (4.7–8.2)	0.40 (0.29–0.54)	<.001	—	—
Sex and risk group							
Male patient with STI	192	1772.7	10.8 (9.4–12.5)	1.00 (referent)		1.00 (referent)	
Hijra (eunuch)	2	4.8	42.0 (5.1–151.6)	3.85 (0.46–14.1)	.11	5.87 (1.44–23.9)	.01
Female patient with STI	14	92.5	15.1 (8.3–25.4)	1.40 (0.75–2.40)	.24	1.26 (0.71–2.22)	.43
Female CSW	9	26.6	33.8 (15.5–64.2)	3.12 (1.41–6.05)	.004	3.90 (1.99–7.62)	<.001
Age group, years							
<20	27	120.3	22.5 (14.8–32.8)	1.00 (referent)		—	—
20–29	133	1198.5	11.1 (9.3–13.2)	0.49 (0.32–0.78)	.002	—	—
⩾30	57	577.8	9.9 (7.5–12.9)	0.56 (0.28–0.73)	<.001	—	—
Education							
None	35	199.6	17.5 (12.2–24.4)	1.00 (referent)		—	—
Less than high school	91	673.4	13.5 (10.9–16.7)	0.77 (0.52–1.17)	.20	—	—
High school or more	91	1021.5	8.9 (7.2–11.0)	0.51 (0.34–0.77)	.001	—	—
Marital status							
Never married	118	996.25	11.8 (9.9–14.3)	1.00 (referent)		—	—
Married	86	832.20	10.3 (8.3–12.9)	0.87 (0.65–1.16)	.34	—	—
Separated[a]	13	68.11	19.1 (10.3–33.2)	1.61 (0.83–2.86)	.12	—	—
Living with family							
Yes	152	1475.0	10.3 (8.8–12.1)	1.00 (referent)		—	—
No	64	417.1	15.3 (11.7–20.0)	1.49 (1.09–2.01)	.01	—	—
No. of sex partners in past 3 months							
None	122	1053.7	11.6 (9.7–13.9)	1.00 (referent)		—	—
1	51	498.6	10.2 (7.6–13.5)	0.88 (0.62–1.23)	.46	—	—
⩾2	43	342.6	12.6 (9.1–16.9)	1.08 (0.75–1.55)	.64	—	—
CSW partners in past 3 months							
No	160	1447.7	11.1 (9.4–12.9)	1.00 (referent)		—	—
Yes	57	448.9	12.7 (9.7–16.6)	1.15 (0.83–1.56)	.37	—	—
Condom use							
No sex partners in past 3 months	139	1352.1	10.3 (8.7–12.2)	1.00 (referent)		—	—
Always	27	227.6	11.9 (7.8–17.3)	1.15 (0.73–1.75)	.49	—	—
Sometimes	14	100.9	13.9 (7.6–23.3)	1.35 (0.72–2.34)	.29	—	—
Never	37	215.9	17.1 (12.1–23.6)	1.67 (1.13–2.41)	.008	—	—
Urethritis/cervicitis at current visit							
No	204	1788.1	11.4 (9.9–13.1)	1.00 (referent)	.83	—	—
Yes	13	108.5	12.0 (6.4–20.5)	1.05 (0.55–1.84)		—	—
Genital lesion at current or previous visit							
None	98	1497.6	6.5 (5.3–8.0)	1.00 (referent)		1.00 (referent)	
Previous visit only	67	224.6	29.8 (23.3–38.1)	4.56 (3.29–6.28)	<.001	3.81 (2.77–5.23)	<.001
Current visit only	15	89.1	16.8 (9.4–27.8)	2.57 (1.39–4.45)	.002	2.73 (1.56–4.79)	<.001
Current and previous visit	36	84.8	42.5 (29.7–58.8)	6.48 (4.30–9.59)	<.001	5.77 (3.91–8.52)	<.001
HIV-1 infection							
No	195	1822.8	10.7 (9.3–12.3)	1.00 (referent)		—	—
Coincident with HSV-2	22	73.4	30.0 (18.8–45.3)	2.80 (1.71–4.36)	<.001	—	—

NOTE. Of 1557 individuals in the analysis, data on level of education were missing for 3; whether participants were living with their families, for 6; no. of sex partners in past 3 months, as reported at a follow-up visit, for 6; presence of genital lesion on examination at follow-up visit, for 2; and HIV-1 status at a follow-up visit, for 1. CI, confidence interval; CSW, commercial sex worker; HIV-1, human immunodeficiency virus type 1.

[a] Individuals who were separated from their spouse, divorced, or widowed.

Table 3. Risk factors for incident human immunodeficiency virus type 1 (HIV-1) infection among patients at 3 sexually transmitted infection (STI) clinics and 1 reproductive tract infection clinic in Pune, India, May 1993 through April 2000.

Characteristic	No. of participants who seroconverted to HIV-1 positive	Person-years	HIV-1 incidence, cases/100 person-years (95% CI)	Unadjusted analysis		Adjusted analysis	
				Rate ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
All participants	224	3867.8	5.8 (5.0–6.6)	—	—	—	—
Screening period, years							
1993–1996	168	2218.8	7.6 (6.5–8.8)	1.00 (referent)		1.00 (referent)	
1997–2000	56	1649.0	3.4 (2.6–4.4)	0.45 (0.32–0.61)	<.001	0.61 (0.43–0.86)	.006
Sex or risk group							
Male patient with STI	165	3249.5	5.1 (4.4–5.9)	1.00 (referent)		1.00 (referent)	
Hijra (eunuch)	3	10.13	29.6 (6.1–86.5)	5.83 (1.19–17.2)	.02	4.61 (1.45–14.6)	.010
Female patient with STI	13	279.39	4.7 (2.5–8.0)	0.92 (0.48–1.61)	.79	0.67 (0.37–1.23)	.20
Female CSW	43	328.74	13.1 (9.5–17.6)	2.58 (1.80–3.62)	<.001	1.00 (0.64–1.58)	.99
Age group, years							
<20	16	174.1	9.2 (5.3–14.9)	1.00 (referent)		1.00 (referent)	
20–29	130	2158.9	6.0 (5.1–7.2)	0.66 (0.39–1.18)	.12	1.01 (0.75–1.36)	.96
⩾30	78	1534.7	5.1 (4.0–6.4)	0.55 (0.32–1.01)	.04	0.63 (0.41–0.99)	.043
Education							
None	73	611.7	11.9 (9.4–15.1)	1.00 (referent)		1.00 (referent)	
Less than high school	88	1453.3	6.1 (4.9–7.5)	0.51 (0.37–0.70)	<.001	0.61 (0.43–0.88)	.007
High school or more	63	1798.9	3.5 (2.7–4.5)	0.29 (0.58–0.79)	<.001	0.38 (0.26–0.57)	<.001
Marital status							
Never married	94	1778.9	5.3 (4.3–6.5)	1.00 (referent)		—	—
Married	95	1752.4	5.4 (4.4–6.7)	1.02 (0.76–1.38)	.86	—	—
Separated[a]	28	318.1	8.8 (5.9–12.8)	1.66 (1.05–2.56)	.02	—	—
Living with family							
Yes	144	2902.6	5.0 (4.2–5.9)	1.00 (referent)		—	—
No	80	959.1	8.3 (6.7–10.4)	1.68 (1.26–2.22)	<.001	—	—
Tattoo in past 3 months[b]							
No	202	3753.4	5.4 (4.7–6.2)	1.00 (referent)		—	—
Yes	13	102.3	12.7 (6.8–21.7)	2.36 (1.24–4.13)	.007	—	—
Medical injection in past 3 months							
No	125	2449.2	5.1 (4.3–6.1)	1.00 (referent)		—	—
Yes	92	1403.6	6.6 (5.3–8.1)	1.28 (0.97–1.69)	.07	—	—
No. of sex partners in past 3 months							
0–1	134	2952.9	4.5 (3.8–5.3)	1.00 (referent)		1.00 (referent)	
>1	89	905.4	9.8 (7.9–12.2)	2.17 (1.64–2.85)	<.001	1.68 (1.22–2.32)	.001
CSW partners in past 3 months							
No	155	3015.3	5.1 (4.4–6.0)	1.00 (referent)		—	—
Yes	69	852.4	8.1 (6.3–10.3)	1.57 (1.17–2.10)	.002	—	—
Condom use in past 3 months							
No sex partners in past 3 months	108	2527.6	4.3 (3.5–5.2)	1.00 (referent)		—	—
Always	32	517.7	6.2 (4.2–8.7)	1.45 (0.94–2.16)	.07	—	—
Sometimes	32	274.9	11.6 (8.0–16.5)	2.72 (1.78–4.07)	<.001	—	—
Never	52	547.5	9.5 (7.1–12.5)	2.22 (1.56–3.12)	<.001	—	—
Urethritis/cervicitis at current visit							
No	202	3649.7	5.5 (4.8–6.4)	1.00 (referent)		—	—
Yes	22	218.0	10.1 (6.3–15.2)	1.82 (1.12–2.84)	.013	—	—
HSV-2 infection							
None	66	1827.9	3.6 (2.8–4.6)	1.00 (referent)		1.00 (referent)	
Prevalent	130	1738.7	7.5 (6.3–8.9)	2.07 (1.53–2.83)	<.001	1.67 (1.22–2.30)	.002
Remote incident	20	265.7	7.5 (4.6–11.6)	2.08 (1.20–3.48)	.007	1.92 (1.15–3.21)	.012
Recent incident	8	35.4	22.6 (9.7–44.5)	6.26 (2.59–13.07)	<.001	3.81 (1.81–8.03)	<.001

NOTE. Of 2732 individuals in the analysis, data on level of education were missing for 4; whether participants were living with their families, for 6; no. of sex partners in past 3 months, as reported at a follow-up visit, for 18; whether participants had a tattoo in the past 3 months, for 30; and whether participant reported at a follow-up visit having received a medical injection in the past 3 months, for 27. CI, confidence interval; CSW, commercial sex worker; HSV-2, herpes simplex virus type 2.

[a] Individuals who were separated from their spouse, divorced, or widowed.

[b] In the past 3 months.

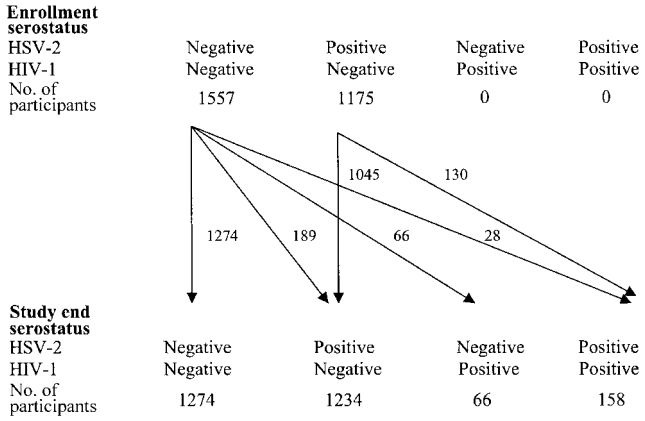

Figure 1. Herpes simplex virus type 2 (HSV-2) and human immunodeficiency virus type 1 (HIV-1) serostatus, at enrollment and at completion of follow-up, in a cohort of patients at 3 sexually transmitted infection clinics and 1 reproductive tract infection clinic in Pune, India, May 1993 through April 2000.

manifestations [22]. Most HSV-2 transmission events between sex partners who are serodiscordant for HSV-2 are not associated with exposure to a sex partner who has experienced a clinically recognized recurrence [27, 28]. Despite the paucity of clinical symptoms associated with HSV-2 infections, frequent shedding of HSV-2 virus has been shown in HSV-2–seropositive individuals [29, 30]. The biological plausibility of an association between HSV-2 and HIV-1 has been explained through the possible generation of a portal of entry by mucosal disruption in the context of active genital ulcer disease. The findings of this study support this hypothesis in the context of prevalent HSV-2 infections, in which we found a significant interaction of clinically apparent or self-reported genital ulcer and serologic evidence of HSV-2 with the risk of HIV-1 acquisition. Individuals in this study who had prevalent HSV-2 infection but no clinically apparent or self-reported genital ulcer were also found to be at increased risk of HIV-1 acquisition, which highlights the importance of asymptomatic infection with HSV-2. The majority of incident HSV-2 infections in this study were asymptomatic, and the presence of a clinically apparent or self-reported genital ulcer did not significantly modify the risk of HIV-1 acquisition. Individuals in this study were not educated about recognition of herpes symptoms, and our findings may, therefore, reflect a lack of awareness of typical symptoms, in addition to some ulcerations that were not clinically amenable to inspection.

Recent in vitro studies offer some insight into the association of HSV-2 with HIV-1 at the cellular level. HSV-2 infection may increase the risk of HIV-1 acquisition through the influx of $CD4^+$ lymphocytes that has been observed in the context of recurrent HSV-2 infection and through the ability of HSV-2 to up-regulate HIV-1 replication [31–33]. The elevated risk of HIV-1 acquisition observed in our study among individuals with exposure to recent incident HSV-2 infections may reflect a more vigorous immune response in individuals who are immunologically naive to HSV-2. Further studies examining the local immune response to incident HSV-2 infection may help explain the elevated risk of HIV-1 acquisition that is associated with exposure to incident HSV-2.

The elevated risk of HIV-1 acquisition among participants with incident HSV-2 infection observed in the present study could result from the exposure of study participants to sex partners who were coinfected with both HSV-2 and HIV-1, leading to the acquisition of both infections simultaneously. Infection with HSV-2 at the mucosal surface could result in higher local HIV-1 shedding, coupled with the increased risk of transmission in the presence of genital ulceration [34–36].

Studies in Zimbabwe and Tanzania also found an association between incident HSV-2 infection and HIV-1 acquisition [18, 19]. Given the overlap in the window periods between exposure and seroconversion of these 2 infections and the possibility that incident HSV-2 infection may pose an independent risk for HIV-1 acquisition, it is not surprising that these infections are most often detected at the same clinic visit, as was observed in these studies. The overlap in seroconversion windows has limited any examination of temporality between incident HSV-2 infection and HIV-1 acquisition. By modeling the follow-up time of HSV-2 exposure as "recent" or "remote," we were able to illustrate a strong relationship between the relative timing of HSV-2 infection and HIV-1 acquisition.

Residual confounding by sexual risk behavior remains a major limitation in any study of the association between HSV-2 and HIV-1. Sexual risk behavior is dynamic in nature, as a result of the different types of exposures that individuals en-

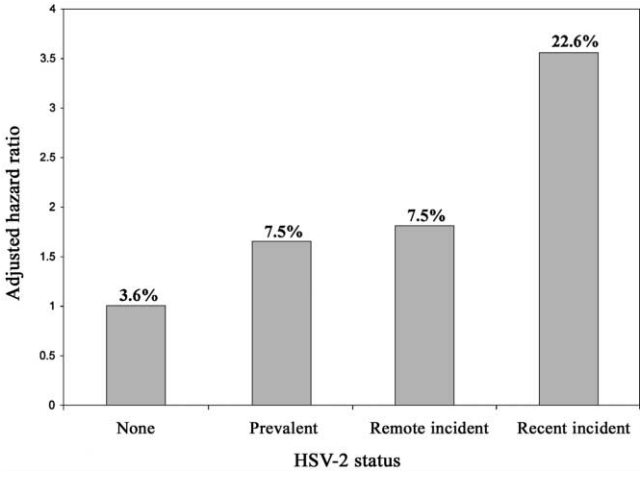

Figure 2. Risk of human immunodeficiency virus type 1 (HIV-1) acquisition, by herpes simplex virus type 2 (HSV-2) infection status, in a cohort of patients at 3 sexually transmitted infection clinics and 1 reproductive tract infection clinic in Pune, India, May 1993 through April 2000. HIV-1 incidence per 100 person-years is given above each column.

rolled in a cohort study may experience over the duration of the study. All changing risk behaviors in this study were treated as time-dependent exposures at each visit, to account for the changing exposure profiles over time and minimize residual confounding.

There are a number of potential limitations in our observation of an association between HSV-2 infection and HIV-1 acquisition. Differential follow-up rates between high-risk individuals (those with commercial sex worker contacts and greater lifetime numbers of sex partners) and low-risk individuals could result in bias. Participants in this study who had a greater lifetime number of sex partners had a longer median follow-up time but were less likely to report for the quarterly follow-up visit. The use of qualitative methods to assess sexual risk behavior relies on the assumption that participants will accurately report on topics that are not openly discussed in India. The counselors conducting the interviews in this study had extensive training in counseling methods, in an attempt to minimize error in the measurement of sexual risk behaviors. There may be a component of misclassification bias in the remote and recent incident HSV-2 categories; some participants who seroconverted to HSV-2 positive and who did not have a documented negative HSV-2 test in the last 6 months may have been classified as having remote, rather than recent, infection. Participants who seroconverted to HIV-1 positive were more likely to have delayed follow-up visits (>7 months), which could result in bias and lead to the finding of a lower risk of HIV-1 infection in association with remote incident HSV-2 exposure than was truly present in our study.

The HSV-2 EIA used in our study has been shown to be 100% sensitive and 96% specific, compared with a Western blot assay [37]. Some of the exposure due to HSV-2 might, therefore, represent false-positive laboratory tests. However, technicians who were blinded to the HIV-1 status of the participant performed all testing, and, therefore, any misclassification in HSV-2 status would be nondifferential and would result in an underestimation of the true association between HSV-2 infection and HIV-1 acquisition.

Two large population-based studies in Mwanza, Tanzania, and Rakai, Uganda, have yielded mixed results on the impact of STI treatment on HIV-1 transmission [38–40]. One hypothesis raised by the authors of the Mwanza and Rakai studies to explain the discrepancy in the outcomes of these 2 trials is that the high rate of untreated HSV infections in Rakai, compared with that in Mwanza, may partially account for the lack of impact of STI control on HIV-1 infection rates [40]. Future studies designed to evaluate the impact of suppressive antiviral therapy on individuals with prevalent HSV-2 infection may help clarify this controversy.

The study of HSV-2 and HIV-1 infection in India adds an important component to the investigation of the interaction between these 2 viral infections. We found that a strong association exists between recently acquired HSV-2 and the risk of HIV-1 acquisition. If an effective vaccine against HSV-2 were available, a targeted approach to primary prevention of HSV-2 in young attendees of STI clinics could have a major impact on the risk of HIV-1 infection for these patients. Suppression of prevalent HSV-2 using antiviral agents may also have an impact on the risk of HIV-1 acquisition in contexts in which the prevalence of HSV-2 infection is high.

The HIV-1 epidemic in India is now 16 years old and has spread rapidly across the country; as a result, India has the potential to have more HIV-1–infected individuals than any country in the world [41]. Little was known, before the present study, about the impact of genital herpes on the HIV-1 epidemic in India. High HSV-2 prevalence and incidence rates were found among patients of STI and reproductive tract infection clinics. The elevated risk of HIV-1 acquisition associated with exposure to recent HSV-2 infection found in our study, coupled with the growing body of evidence that prevalent HSV-2 plays a major role in HIV transmission, provides a strong argument for the prioritization of HSV-2 vaccine development and other HSV-2 prevention strategies as key components of the current global HIV prevention research agenda.

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References

1. Stamm WE, Handsfield HH, Rompalo AM, Ashley RL, Roberts PL, Corey L. The association between genital ulcer disease and acquisition of HIV infection in homosexual men. JAMA **1988**; 260:1429–33.
2. Mehendale SM, Rodrigues JJ, Brookmeyer RS, et al. Incidence and predictors of human immunodeficiency virus type 1 seroconversion in patients attending sexually transmitted disease clinics in India. J Infect Dis **1995**; 172:1486–91.
3. Bollinger RC, Brookmeyer RS, Mehendale SM, et al. Risk factors and clinical presentation of acute primary HIV infection in India. JAMA **1997**; 278:2085–9.
4. Telzak EE, Chiasson MA, Bevier PJ, Stoneburner RL, Castro KG, Jaffe HW. HIV-1 seroconversion in patients with and without genital ulcer disease: a prospective study. Ann Intern Med **1993**; 119:1181–6.
5. Plummer FA, Wainberg MA, Plourde P, et al. Detection of human immunodeficiency virus type 1 (HIV-1) in genital ulcer exudate of HIV-1–infected men by culture and gene amplification. J Infect Dis **1990**; 161: 810–1.
6. Plummer FA, Simonsen JN, Cameron DW, et al. Cofactors in male-female sexual transmission of human immunodeficiency virus type 1. J Infect Dis **1991**; 163:233–9.
7. Beyrer C, Jitwatcharanan K, Natpratan C, et al. Molecular methods for the diagnosis of genital ulcer disease in a sexually transmitted disease clinic population in northern Thailand: predominance of herpes simplex virus infection. J Infect Dis **1998**; 178:243–6.
8. Risbud A, Chan-Tack K, Gadkari D, et al. The etiology of genital ulcer

disease by multiplex polymerase chain reaction and relationship to HIV infection among patients attending sexually transmitted disease clinics in Pune, India. Sex Transm Dis **1999**; 26:55–62.
9. Chen CY, Ballard RC, Beck-Sague CM, et al. Human immunodeficiency virus infection and genital ulcer disease in South Africa: the herpetic connection. Sex Transm Dis **2000**; 27:21–9.
10. Morse SA, Trees DL, Htun Y, et al. Comparison of clinical diagnosis and standard laboratory and molecular methods for the diagnosis of genital ulcer disease in Lesotho: association with human immunodeficiency virus infection. J Infect Dis **1997**; 175:583–9.
11. Behets FM-T, Andriamiadana J, Randrianasolo D, et al. Chancroid, primary syphilis, genital herpes, and lymphogranuloma venereum in Antananarivo, Madagascar. J Infect Dis **1999**; 180:1382–5.
12. Limpakarnjanarat K, Mastro TD, Saisorn S, et al. HIV-1 and other sexually transmitted infections in a cohort of female sex workers in Chiang Rai, Thailand. Sex Transm Infect **1999**; 75:30–5.
13. Gwanzura L, McFarland W, Alexander D, Burke RL, Katzenstein D. Association between human immunodeficiency virus and herpes simplex virus type 2 seropositivity among male factory workers in Zimbabwe. J Infect Dis **1998**; 177:481–4.
14. Hook EW III, Cannon RO, Nahmias AJ, et al. Herpes simplex virus infection as a risk factor for human immunodeficiency virus infection in heterosexuals. J Infect Dis **1992**; 165:251–5.
15. Nelson KE, Eiumtrakul S, Celentano D, et al. The association of herpes simplex virus type 2 (HSV-2), *Haemophilus ducreyi,* and syphilis with HIV infection in young men in northern Thailand. J Acquir Immune Defic Syndr Hum Retrovirol **1997**; 16:293–300.
16. Wald A, Link K. Risk of human immunodeficiency virus infection in herpes simplex virus type 2–seropositive persons: a meta-analysis. J Infect Dis **2002**; 185:45–52.
17. Holmberg SD, Stewart JA, Gerber AR, et al. Prior herpes simplex virus type 2 infection as a risk factor for HIV infection. JAMA **1988**; 259: 1048–50.
18. Mar Pujades RM, Obasi A, Mosha F, et al. Herpes simplex virus type 2 infection increases HIV incidence: a prospective study in rural Tanzania. AIDS **2002**; 16:451–62.
19. McFarland W, Gwanzura L, Bassett MT, et al. Prevalence and incidence of herpes simplex virus type 2 infection among male Zimbabwean factory workers. J Infect Dis **1999**; 180:1459–65.
20. Corey L, Handsfield HH. Genital herpes and public health: addressing a global problem. JAMA **2000**; 283:791–4.
21. Fleming DT, McQuillan GM, Johnson RE, et al. Herpes simplex virus type 2 in the United States, 1976 to 1994. N Engl J Med **1997**; 337: 1105–11.
22. Langenberg AG, Corey L, Ashley RL, Leong WP, Straus SE. A prospective study of new infections with herpes simplex virus type 1 and type 2. Chiron HSV Vaccine Study Group. N Engl J Med **1999**; 341:1432–8.
23. Siegel D, Golden E, Washington AE, et al. Prevalence and correlates of herpes simplex infections: the Population-Based AIDS in Multiethnic Neighborhoods Study. JAMA **1992**; 268:1702–8.
24. Centers for Disease Control and Prevention. Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. MMWR Morb Mortal Wkly Rep **1989**; 38(Suppl 7):1–7.
25. Hosmer DW, Lemeshow S. Applied logistic regression. 2nd ed. New York: Wiley, **2000**.
26. Breslow NE, Day NE. Statistical methods in cancer research. Vol 2. The design and analysis of cohort studies. New York: International Agency for Research on Cancer, **1987**.
27. Mertz GJ, Schmidt O, Jourden JL, et al. Frequency of acquisition of first-episode genital infection with herpes simplex virus from symptomatic and asymptomatic source contacts. Sex Transm Dis **1985**; 12: 33–9.
28. Mertz GJ, Benedetti J, Ashley R, Selke SA, Corey L. Risk factors for the sexual transmission of genital herpes. Ann Intern Med **1992**; 116: 197–202.
29. Krone MR, Wald A, Tabet SR, Paradise M, Corey L, Celum CL. Herpes simplex virus type 2 shedding in human immunodeficiency virus–negative men who have sex with men: frequency, patterns, and risk factors. Clin Infect Dis **2000**; 30:261–7.
30. Wald A, Corey L, Cone R, Hobson A, Davis G, Zeh J. Frequent genital herpes simplex virus 2 shedding in immunocompetent women: effect of acyclovir treatment. J Clin Invest **1997**; 99:1092–7.
31. Cunningham AL, Turner RR, Miller AC, Para MF, Merigan TC. Evolution of recurrent herpes simplex lesions: an immunohistologic study. J Clin Invest **1985**; 75:226–33.
32. Koelle DM, Abbo H, Peck A, Ziegweid K, Corey L. Direct recovery of herpes simplex virus (HSV)–specific T lymphocyte clones from recurrent genital HSV-2 lesions. J Infect Dis **1994**; 169:956–61.
33. Moriuchi M, Moriuchi H, Williams R, Straus SE. Herpes simplex virus infection induces replication of human immunodeficiency virus type 1. Virology **2000**; 278:534–40.
34. Gadkari DA, Quinn TC, Gangakhedkar RR, et al. HIV-1 DNA shedding in genital ulcers and its associated risk factors in Pune, India. J Acquir Immune Defic Syndr Hum Retrovirol **1998**; 18:277–81.
35. Schacker T, Ryncarz AJ, Goddard J, Diem K, Shaughnessy M, Corey L. Frequent recovery of HIV-1 from genital herpes simplex virus lesions in HIV-1–infected men. JAMA **1998**; 280:61–6.
36. Mbopi-Kéou F-X, Grésenguet G, Mayaud P, et al. Interactions between herpes simplex virus type 2 and human immunodeficiency virus type 1 infection in African women: opportunities for intervention. J Infect Dis **2000**; 182:1090–6.
37. Prince HE, Ernst CE, Hogrefe WR. Evaluation of an enzyme immunoassay system for measuring herpes simplex virus (HSV) type 1–specific and HSV type 2–specific IgG antibodies. J Clin Lab Anal **2000**; 14:13–6.
38. Grosskurth H, Mosha F, Todd J, et al. Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial. Lancet **1995**; 346:530–6.
39. Wawer MJ, Sewankambo NK, Serwadda D, et al. Control of sexually transmitted diseases for AIDS prevention in Uganda: a randomised community trial. Rakai Project Study Group. Lancet **1999**; 353:525–35.
40. Grosskurth H, Gray R, Hayes R, Mabey D, Wawer M. Control of sexually transmitted diseases for HIV-1 prevention: understanding the implications of the Mwanza and Rakai trials. Lancet **2000**; 355:1981–7.
41. Joint United Nations Programme on HIV/AIDS (UNAIDS)/World Health Organization (WHO). AIDS epidemic update—December 2001. Geneva: UNAIDS/WHO, **2001**. Available at: http://www.who.int/hiv/facts/isbn9291731323.pdf. Accessed on: 18 April 2002.