The Impact of Incident and Prevalent Herpes Simplex Virus-2 Infection on the Incidence of HIV-1 Infection Among Commercial Sex Workers in South Africa

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Summary: This study investigated the impact of prevalent and incident HSV-2 infection on the incidence of HIV-1 infection in a cohort of female commercial sex workers in KwaZulu-Natal, South Africa. Prior to a vaginal microbicide trial, 416 women were screened for antibodies to HIV-1 and herpes simplex virus-2 (HSV-2) infections and a questionnaire was used to establish behavioral, social, and demographic characteristics. A total of 187 HIV-1-seronegative women were followed up at monthly intervals when blood was drawn and used to detect HIV-1 and HSV-2 antibodies. The median duration of follow-up was 2.2 years. At screening 50% of the women were HIV-1 seropositive and 84% were HSV-2 seropositive. The hazards of HIV-1 among women who were HSV-2 seropositive or seronegative throughout, or among those who seroconverted during the study, were not significantly different. When HSV-2 seroconversion was analyzed as a time-dependent covariate, the hazard ratio for HIV-1 seroconversion was 6.0 (95% CI: 2.6–14.0) times greater among women with incident than among women with prevalent HSV-2 infections. Drawing on other recent studies these data suggest that incident HSV-2 infection increases the risk of HIV-1 infection; the effect wanes with time since infection; and the effect is significantly greater for men than it is for women.

Key Words: HIV, herpes simplex virus, AIDS, incidence, South Africa, sex worker

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Herpes simplex virus type II (HSV-2) infection is the most common cause of genital ulcer disease in the world[1,2] and may increase the risk of HIV-1 transmission to women through disruption and inflammation of the epithelial barrier.[3] Recent studies reveal that even in the absence of genital ulcer disease, there is a correlation between genital tract shedding of HIV-1 and HSV-2.[4,5] In sub-Saharan Africa, where HIV-1 infection is spread mainly by heterosexual transmission, other sexually transmitted infections, including HSV-2 infection, are common.[6–8] In South Africa in 1994, 50% of men attending sexually transmitted disease clinics were infected with HSV-2, which was the most common cause of genital ulcer disease,[9] while a community survey carried out in 1999 in Carletonville found that 89% of women and 42% of men were infected with HSV-2 by the time they reached 25 years of age.[10] Among sex workers and other high-risk groups, high rates of both HSV-2 and HIV-1 infections have been reported in Africa and elsewhere.[11,12]

Several studies have found a positive association between HSV-2 infection and HIV-1 infection.[7,13–15] In a study conducted in 4 African cities, the adjusted odds ratio for prevalent HIV-1 and HSV-2 infection among women ranged from 4.0 in Kenya to 5.5 in Yaounde.[12] In this paper we estimate the extent to which prevalent HSV-2 infections increase the risk of acquiring HIV-1 infections among a group of female commercial sex workers in KwaZulu-Natal, South Africa, and whether this risk is increased further for women with recently acquired HSV-2 infections. In 3 studies[1,16,17] estimates have been made of the hazard or odds ratio for HIV-1 in people who were HSV-2 positive compared with people who were HSV-2 negative throughout the study (we refer to this as the hazard or odds ratio for prevalent infection) and the hazard or odds ratio for HIV-1 in people who seroconverted to HSV-2 during the study, regardless of when they seroconverted, as compared with people who were HSV-2 negative throughout the study (we refer to this as the "hazard or odds ratio for incident infection").

In the first study 2397 male factory workers in Zimbabwe[16] were followed up every 6 months for 4 years. HSV-2 incidence was 6.2% per year while HIV-1 incidence was about 2.5% per year. The adjusted HIV-1 hazard ratios for prevalent and incident HSV-2 infection were 3.5 (2.2–5.8; unless otherwise stated errors are 95% CIs) and 6.7 (4.2–10.7), respectively. The adjusted HSV-2 hazard ratios for prevalent and incident HIV-1 infection were 4.7 (3.3–6.7) and 3.9 (2.6–5.8), respectively. There was no evidence that either infection was more or less likely to precede the other.

In the second, case-control study in rural Tanzania[17] with 70 male and 57 female cases (and 636 controls), HSV-2 incidence was about 10% per year (but 20% per year in women <20 years old) while HIV-1 incidence was about 0.75% per year.[18] Among men the adjusted HIV-1 odds ratios for

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prevalent and incident HSV-2 infection were 6.1 (2.5–14.9) and 16.8 (6.1–46.3), respectively. These rates were higher than, but not significantly different from, the hazard ratios found in Zimbabwe. For women the effect of HSV-2 infection on the incidence of HIV-1 was not significant and the corresponding adjusted odds ratios were 1.3 (0.6–2.8) and 2.4 (0.8–6.8), respectively.

In the 3rd study in Pune, India,[1] 2269 male patients with sexually transmitted infection, 9 of whom were "hijra" (eunuchs), and 463 women who were partners of male sexually transmitted infection (STI) patients, commercial sex workers, or had reproductive tract infections, were followed up 3 times over 11 months (median values). The HSV-2 incidence was 11.4% (9.9%–13.0%) per year while the HIV-1 incidence was 5.8% (5.0%–6.6%) per year. In this study, which included mainly men, the HIV-1 adjusted hazard ratios for prevalent and incident HSV-2 infection were 1.7 (1.2–2.3) and 2.7 (1.7–4.3), respectively. (The latter estimate is the weighted average of "recent" and "remote" incident infections as given by the authors). In all 3 studies, the HIV-1 hazard or odds ratios for HSV-2 incident infection were greater than for HSV-2 prevalent infection. However, in none of these studies were the differences in the hazard or odds ratios significant. Furthermore, in the one study for which results were reported separately for women,[17] neither odds ratio differed significantly from 1.

Here we investigate the impact of prevalent and incident HSV-2 infection on the incidence of HIV-1 infection in a cohort of female sex workers who were followed up and tested monthly for antibodies to HIV-1 and HSV-2. The HSV-2 incidence was 35% per year while the HIV-1 incidence was 18% per year. The longest follow-up was for 3.6 years (median 2.2 years). In addition we do an analysis with HSV-2 seroconversion treated as a time-dependent covariate. We provide a comparative analysis of the previously published studies to determine more precisely the hazard ratio for HIV-1 incidence among people with prevalent and incident HSV-2 infections.

STUDY POPULATION AND METHODS

A total of 416 sex workers from 5 truck stops between the port city of Durban and the commercial center of Johannesburg, South Africa, were invited to participate in a multicenter vaginal microbicide (Advantage S: Columbia Laboratories, Paris, France, containing 52.5 mg nonoxynol-9, N9) phase 3 clinical trial funded by UNAIDS, which has been described in detail elsewhere.[19]

Of the 416 women screened, 208 were excluded because they were already HIV-1 positive. Because the original study was a trial of a microbicide that was still undergoing reproductive toxicology trials, and in which condoms were promoted, a further 12 women were excluded because they had <5 sexual partners per week, were pregnant or planning to become pregnant, or were allergic to latex. At enrollment the 196 eligible HIV-negative women were educated in HIV-1 prevention, counseled on safe sexual behavior, and randomly assigned to either the N9 or the placebo arm.[20] Nine women did not return after the enrollment visit and were not included in the survival analysis. During each follow-up visit the women were counseled and told that other STIs may increase their likelihood of being infected with HIV, and blood was drawn to test for HIV-1 infections. The HSV-2 tests were done retrospectively after the main study had been completed. A clinician gave each woman a gynecologic examination and took swabs to test for *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and candidiasis. STIs were treated according to the South African syndromic management guidelines.

At each follow-up visit, the women completed a questionnaire on sexual behavior, were provided with condoms, and were counseled to use them whenever they had sex. The study was approved by the University of Natal Ethics Committee and the agencies that funded the initial trial.

Laboratory Tests

Blood specimens were tested for HIV-1 infection using a highly sensitive antibody enzyme-linked immunosorbent assay (ELISA) (Vironostika) and positive specimens were retested using a 2nd highly specific ELISA (Abbott). Recently acquired syphilis was determined using an rapid plasma reagin (RPR) test confirmed with treponema pallidium haemagglutination assay/fluorescent treponemal antibody test (TPHA/FTA) tests. *T. vaginalis* and candidiasis were tested by wet mount. Endocervical swabs were taken and tested for *C. trachomatis* using an ELISA (Syva). *N. gonorrhoeae* infection was detected by culture. HSV-2 was detected using Gull ELISA (Meridian) for which the sensitivity was 93% and the specificity was 97%.[21,22] All HIV-1 seroconversions were confirmed at the Institute of Tropical Medicine, Antwerp, Belgium. Once the HIV-1 infection was confirmed, sera were tested for HSV-2 infection starting from the date of HIV-1 seroconversion and going back to the first positive HSV-2 test.

Statistical Analysis

Analyses were done using Stata (Stata Corporation, College Station, TX) (Release 8). Kaplan-Meier survival curves for the time to HIV-1 seroconversion were calculated for various groups of women and we estimated crude incidence rates for each group. Three Cox proportional hazards models were used to determine hazard ratios for HIV-1 seroconversion and to allow for confounding variables. Model I was used to test potential confounding variables for inclusion in subsequent models. Model II was used to test for the effect of HSV-2 admission status on entry and exit while adjusting for confounders. Model III was used to test for the effect of recent HSV-2 seroconversion by including it as a time-dependent variable in the Cox proportional hazards model. Schoenfeld residuals were used to test the assumption of proportional hazards.

RESULTS

The prevalence of both HIV-1 and HSV-2 among these women was high. On recruitment, 50% of 416 women tested positive for HIV-1 infection and 84% for HSV-2 infection, and HIV-1 and HSV-2 serostatus were strongly associated (odds ratio = 4.6, 2.5–8.3). As in other populations in South Africa, the prevalence of HIV-1 increases with age, peaks among women at the age of about 24 years, and declines with age

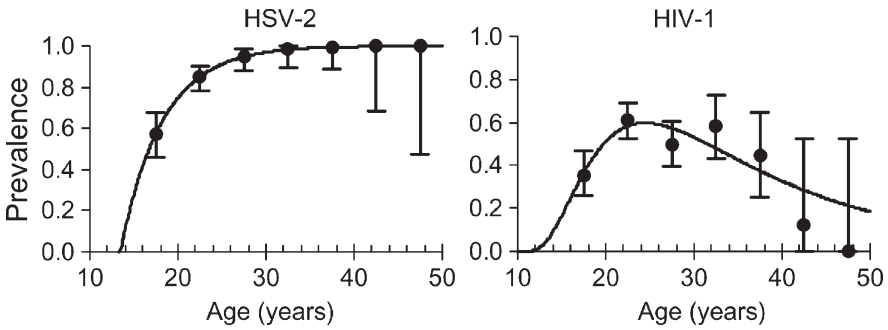

FIGURE 1. Age-specific prevalence of HSV-2 (left) and HIV-1 (right) infection for women at recruitment. The HSV-2 data are fitted to an exponential curve. The HIV-1 data are fitted to a log-normal curve.

thereafter.[23] The prevalence of HSV-2 infection, conversely, increases rapidly with age, reaching 60% at the age of 18 years, 90% at the age of 25 years, and 100% by the age of 35 years (Fig. 1).

Baseline data for potential risk factors and the prevalence of HSV-2 and HIV-1 are given in Tables 1 and 2. Education levels were reasonably high; 49% of the women had 4–7 years of education while a further 39% had ≥8 years of education. Reported condom use was low and 41% of women said that they never used condoms, while only 11% said that they always used condoms. Forty percent of women had engaged in anal sex. Curable sexually transmitted infections were common, with 31% testing positive for syphilis, 10% for *N. gonorrhoeae*, 12% for *C. trachomatis*, and 36% for *T. vaginalis* infection. The average age of the women was 25 years; they had an average of 20 partners per week and had been doing sex work for an average of 2.5 years. Of the categorical variables (Table 1), syphilis was strongly associated with HSV-2 infection ($P = 0.004$) while *C. trachomatis* infection was weakly associated with HSV-2 infection ($P = 0.059$). Of the continuous variables (Table 2), age was significantly associated with HSV-2 status ($P = 0.001$).

TABLE 1. Prevalence of HSV-2 and HIV-1 and the Proportion of Women in Potential Risk Groups

Risk Factor	χ^2	%	HSV-2 (%)	*P*	HIV-1 (%)	*P*
Education (y)	0–3	12.5	83.7		44.9	
n = 391	4–7	48.8	84.3		47.1	
	8+	38.6	84.1	0.994	57.6	0.104
Condom use	Never	40.6	83.2		45.5	
n = 411	Sometimes	40.9	85.7		52.9	
	Often	7.3	93.3		50.0	
	Always	11.2	78.3	0.316	54.4	0.515
Anal sex	Yes	40.0	86.7		49.7	
n = 412	No	60.0	83.0	0.314	50.2	0.920
Syphilis	Yes	31.4	92.7		54.8	
n = 395	No	68.6	81.5	0.004	48.3	0.231
Gonorrhoea	Yes	10.3	90.0		60.0	
n = 387	No	89.7	85.0	0.396	49.6	0.211
T. vaginalis	Yes	35.7	89.3		50.7	
n = 392	No	64.3	84.1	0.159	51.2	0.928
C. trachomatis	Yes	11.8	95.1		48.8	
n = 347	No	88.2	83.9	0.059	50.3	0.852

P values are for χ^2 tests on each potential risk factor at screening.

Figure 2A shows Kaplan-Meier curves for time to HIV seroconversion for women who were HSV-2 positive throughout (PP), HSV-2 negative throughout (NN), and HSV-2 negative on entry and positive on exit (NP). During the study period 24 women seroconverted to HSV-2 and, of these, 20 seroconverted to HIV-1 after HSV-2; 3 seroconverted in the same time interval; and 1 seroconverted to HIV-1 before HSV-2. Table 3 gives the crude HIV-1 incidences for each group (PP, NN, NP) and these were not significantly different. When time was measured from HSV-2 seroconversion, the incidence of HIV-1 was significantly greater than in those who were HSV-2 positive on entry.

Table 4 shows the results of a univariate analysis for potential risk factors for HSV-2 infection; only age and syphilis were significantly associated with HSV-2 status at entry. Cox proportional hazards models were then used to determine HIV-1 hazard ratios for the different groups of women according to their HSV-2 status. In model I (Table 5), the HIV-1 hazard ratio was assessed in relation to HSV-2 status at entry on its own and then in combination with each potential confounding variable in a series of bivariate analyses; only "study group" is close to being significant ($P = 0.078$). The effect of HSV-2 status on HIV-1 incidence remained significant when combined with each of the other variables except in the case of age, but then age was itself not significant. The hazard ratio for HSV-2 status on entry was little changed by the inclusion of each of the other variables.

In model II (Table 6) the women were categorized into 3 groups: those who were HSV-2 seropositive on admission, HSV-2 seronegative throughout, and HSV-2 seronegative on admission but seropositive on exit. All variables with a *P* value <0.2 in model I (study group, duration of sex work, and gonorrhoea) were included in a multivariate model to assess their combined effect on HIV-1 incidence. Since both age and syphilis were significantly associated with the HSV-2 infection status of the women (Table 4), these were initially included, but neither age nor syphilis was then significant ($P = 0.775$ and 0.419, respectively) and they were omitted from the final model. It is worth noting that the women included in the final analysis covered a narrow age range (median 24 years; interquartile range 19–29 years). In this analysis women with prevalent infections (PP), ie, those who were positive throughout the study, were taken as the reference group both because it was the largest group and because we wished to see whether women with incident infections are at significantly greater risk than women with prevalent infections. Model II

TABLE 2. Mean Values With 95% CIs and *P* Values for Potential Risk Factors for HIV-1 and HSV-2 Infection at Screening

	HIV-1 Positive (n = 206)	HIV-1 Negative (n = 206)	*P*	HSV-2 Positive (n = 348)	HSV-2 Negative (n = 64)	*P*
Age (y)	24.8 ± 5.3	25.1 ± 7.0	0.620	25.8 ± 6.2	19.9 ± 3.6	0.001
Partners/wk	18.9 ± 8.9	19.5 ± 8.0	0.501	18.9 ± 8.4	20.6 ± 9.0	0.167
Months of sex work	26.7 ± 23.8	31.9 ± 37.2	0.093	30.1 ± 32.5	25.0 ± 22.8	0.129

(Table 6) shows that of women who remained negative throughout (NN) or who seroconverted during the study (NP) had hazard ratios that were greater than for women who were positive on entry (PP); in neither case was the difference significant.

Finally, in model III we proceeded as for model II but we treated HSV-2 seroconversion as a time-dependent covariate. In this analysis we combine all people who are HSV-2 negative up to the time when they either seroconvert to HSV-2 or leave the study in a group that we call NT. We then consider all those who seroconvert during the study in a 2nd group and measure HIV-1 incidence from the time of HSV-2 seroconversion and we call this group PT. The statistical comparison group is, again, PP, those who were HSV-2 positive throughout. Kaplan-Meier curves for these 3 groups are given in Figure 2B. In this analysis (Table 6), the hazard ratio for HIV-1 seroconversion for women who were HSV-2 negative (NT) was greater than for women who were HSV-2 positive throughout (PP), but again the difference was not significant. However, the hazard ratio for HIV-1 seroconversion in women after they seroconverted to HSV-2 (PT) is now 6.0 (2.6–14.0) times that for women who remained positive throughout (PP).

The data suggest, therefore, that immediately after HSV-2 seroconversion women experience a substantial, and statistically significant, increase in the risk of acquiring HIV-1 (when HSV-2 incidence is included as a time-dependent variable; model III in Table 6). Conversely, the data do not provide conclusive evidence for differences between the HIV-1 hazard ratio for what we have defined above as prevalent and incident HSV-2 infection (ie, comparing the groups PP, NN, and NP in Table 6, Model II).

Comparisons With Other Studies

The HIV-1 hazard ratios for incident and prevalent HSV-2 infection have been measured in 2 other studies, carried out in Zimbabwe[16] and India,[1] and the corresponding odds ratios have been measured in a study in Tanzania.[17] The results of these studies are shown in Figure 3, where the final model in this paper (model II, Table 6) has been repeated using a logistic regression to determine the odds ratios. In the studies on men[16,17] or mainly on men,[1] both prevalent and incident HSV-2 infection increased the risk of acquiring HIV-1 infection significantly. In the present study (recalculated so that the comparison is with women who remained seronegative throughout) and the study on women in Tanzania,[17] neither prevalent nor incident HSV-2 infection increased the risk of acquiring HIV-1 infection significantly. The data from the India study[1] are for a heterogeneous group of men and women, and if we put this study aside the other 4 results in Figure 3 suggest that HSV-2 has less relative impact on the risk of HIV-1 infection in women than it does in men.

In none of these studies is the HIV-1 hazard ratio or odds ratio significantly different for prevalent and incident HSV-2 infections. However, on a nonparametric binomial test, the probability that 5 results all show that the hazard or odds ratios for incident infections is greater than for prevalent infections is 0.031. Furthermore, the Zimbabwe, India, and South African studies give a weighted average for the HIV-1 hazard ratio for incident as compared with prevalent HSV-2 infection of 1.85 (1.16–2.95; $P = 0.010$) while the Tanzania study and the South African study (using a logistic regression) give a weighted average for the HIV-1 odds ratio for incident as compared with prevalent HSV-2 infection of 2.37 (1.09–5.17; $P = 0.031$).

In a study in San Francisco, incident HIV-1 and HSV-2 infections among men who have sex with men (MSM) were correlated while prevalent infection with either virus was not significantly associated with subsequent infection with the other.[24] However, the median time between clinic visits was 9 months and there were only 11 HSV-2 seroconversions among the MSM, all of whom seroconverted to HIV-1 in the same

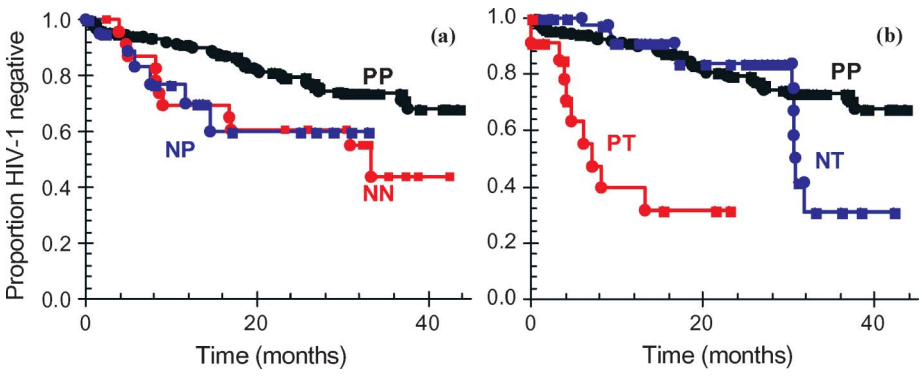

FIGURE 2. Kaplan-Meier curves for HIV-1-free survival. A, Among those who were HSV-2 positive on enrollment (PP); HSV-2 negative throughout (NN); seroconverted to HSV-2 during the course of the trial (NP). B, Among those who were negative until they left the trial or seroconverted to HSV-2 (NT); and from the time at which they seroconverted to HSV-2 (PT). *Dots* indicate times at which women seroconverted; *squares* indicate times at which they were lost to follow-up.

TABLE 3. Crude HIV-1 Incidence for Women According to Their HSV-2 Serostatus

	n	Seroconverters	Person-Years	Incidence (%/y)	95% CI (%/y)
HSV-2 positive at enrolement (PP)	143	35	285	12.3	8.9–16.2
HSV-2 negative at enrolement					
HSV-2 negative at exit (NN)	20	6	23	26.1	10.3–47.2
HSV-2 positive at exit (NP)	24	11	45	24.4	13.6–35.8
Time since HSV-2 seroconversion	20	8	15	53.5	25.8–85.3

For those who were HSV-2 negative at enrollment, the HIV-1 incidence is given separately for those who remained HSV-2 negative throughout (NN), for those who became HSV-2 positive (NP), and from time since HSV-2 seroconversion.

period. While the results of this study are consistent with the results reported, here the temporal sequence of events could not be established and the statistical power was low.

DISCUSSION

Many studies have shown a strong positive association between infection with HSV-2 and HIV-1; a direct causal link is more difficult to establish. Nevertheless, if the presence of either infection increases the incidence of the other it is likely that in populations in which HSV-2 is common HIV-1 will also spread rapidly, and it has been suggested that this might explain some of the regional variation in HIV-1 infection rates in sub-Saharan Africa.[17] Some studies have found that HSV-2 infection increases HIV-1 incidence in men[1,16,17] but not in women[17] and in one study the HIV-1 hazard ratio for incident or prevalent HSV-2 infection is between about 4–5.[16] All of these studies suggest that the impact of HSV-2 on HIV-1 is greater for those with incident than for those with prevalent HSV-2 infection, but in none of these studies is the difference statistically significant. Here we present data to show that the incidence of HIV-1 infection is significantly greater in women with incident HSV-2 infection (when HSV-2 incidence is analyzed as a time-dependent variable) than it is in women with prevalent HSV-2 infection. Furthermore, we are able to show that if we combine the results from all the studies, the hazard or odds ratios for HIV-1 infection is about twice as great in people with incident as opposed to prevalent infections (where incident infections refer to people who seroconverted during the study) and that this difference is then statistically significant.

The studies discussed in this paper cover a wide range of settings including male factory workers in Zimbabwe, men and women in rural Tanzania, a mixture of men and women with STIs in India, and commercial sex workers in South Africa. The background rates of infection also vary

TABLE 4. Total Number (n) of Women in Each Comparison (numbers differ because some data are missing) and Percentage of These in the Different Levels of Potential Risk Factors for HSV-2

		HSV-2						
		Negative Throughout		Negative Entry, Positive Exit		Positive on Entry		
Factor	Level	n	%	n	%	n	%	*P*
Age (y)	<20 (*cf* > 20)	20	70	21	67	152	15	0.001
Education (y)	<8 (*cf* > 8)	19	63	23	57	139	65	0.749
Study group	N9 (*cf* placebo)	20	65	24	54	149	47	0.286
Condom Use								
Never		20	30	24	55	152	46	0.583
Sometimes			45		29		37	
Often			35		17		17	
Type of sex								
Anal sex (*cf* Not)		20	45	24	38	152	42	0.873
Oral sex (*cf* Not)		20	40	24	38	152	31	0.620
Sexually transmitted infections								
Syphilis		19	11	23	9	147	33	0.010
Gonorrhoea		19	5	21	10	145	11	0.733
T. vaginalis		19	26	20	25	147	38	0.351
C. trachomatis		16	6	20	5	134	16	0.250
Sex work	Months (median IQR)	22	9–34	23	9–32	24	17–36	0.125
Partners	Per wk (median IQR)	20	12–28	20	11–28	20	14–25	0.730

The last 2 rows give the median and the interquartile range for continuous variables.

TABLE 5. Model I: Cox Proportional Hazards models for HIV-1 Seroconversion

Additional Variable	HSV-2		Additional Variable	
	Hazard Ratio	*P*	Hazard Ratio	*P*
HSV-2 only	0.460	0.0091		
Age	0.539	0.0744	0.742	0.3674
Education	0.481	0.0164	0.808	0.4521
Study group	0.497	0.0200	1.671	0.0778
Condom use	0.463	0.0099	1.127	0.7350
Anal sex	0.464	0.0099	0.819	0.4898
Oral sex	0.465	0.0103	1.204	0.5211
Syphilis	0.474	0.0174	1.119	0.7293
Gonorrhoea	0.422	0.0053	1.777	0.1391
Chlamydia	0.418	0.0064	1.329	0.4712
Duration sex work	0.519	0.0325	0.992	0.1880
Partners/wk	0.451	0.0076	0.983	0.3288

The hazard ratio is given for HSV-2 on its own and when combined with each potential confounding variable. "Study group" refers to N9 or placebo during the original trial. "Age" was categorized into those < or > 20 years old; condom use into those who never or sometimes used condoms and those who always used condoms; duration of sex work was analyzed as a continuous variable.

substantially. The incidence of HSV-2 is lower in Zimbabwe (~6% per year) but much higher in South Africa (~35% per year) than it is in India or Tanzania (~10% per year). The incidence of HSV-2 is 2–3 times greater than the incidence of HIV-1 in all studies except in Tanzania, where it is about 7 times greater. Nevertheless, a consistent picture emerges of the impact on men and women and when comparing incident and prevalent HSV-2 infections.

TABLE 6. Cox Proportional Hazards Models for HIV-1 Seroconversion

Model	Risk Factor	Level	Hazard Ratio	95% CI	*P*
II	HSV-2	PP	1		
		NN	1.77	0.68–4.66	0.243
		NP	1.94	0.95–3.98	0.071
	Study group	Placebo	1		
		N9	1.79	0.97–3.29	0.063
	Years of sex work	Per year	0.86	0.72–1.02	0.089
	Gonorrhoea	Negative	1		
		Positive	1.83	0.85–3.97	0.124
III	HSV-2	PP	1		
		NT	1.80	0.68–4.73	0.235
		PT	5.98	2.57–13.95	0.0001
	Study group	Placebo	1		
		N9	1.71	0.91–3.24	0.097
	Years of sex work	Per year	0.89	0.76–1.05	0.156
	Gonorrhoea	Negative	1		
		Positive	1.92	0.84–4.42	0.124

Model II shows the effect of HSV-2 status on entry and exit, model III the effect of HSV-2 analyzed as a time-dependent covariate, both models adjusted for potential confounders. PP indicates positive on entry and exit; NN negative on entry and exit, NP negative on entry, positive on exit. NT and PT refer to women before and after HSV-2 seroconversion when HSV-2 is treated as a time-dependent variable.

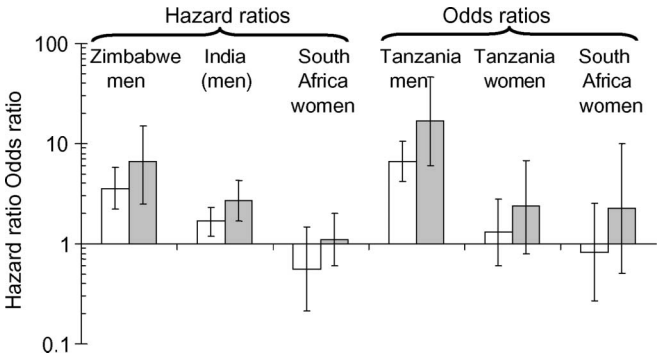

FIGURE 3. The hazard or odds ratios for HIV-1 incidence (plotted on a logarithmic scale) when comparing people with prevalent (*white bars*) and incident (*gray bars*) HSV-2 infections to people who remained HSV-2 negative throughout the studies. Details of the studies are given in the text.

In this study women were followed up every month, while they were either followed up at approximately 3-monthly intervals in the Indian study,[17] approximately 6-monthly intervals in the Zimbabwe study,[16] and after 2 years in the Tanzanian case-control study.[17] The study reported here is the only one in which the follow-up interval is short enough to analyze incident HSV-2 seroconversions as a time-dependent covariate.

This study has some limitations. The small sample size limits the power of the study and the study was a retrospective study, relying on data collected for another purpose. Nevertheless, the results are statistically significant and consistent with previous studies. While age may be a confounder, the age range of the women was narrow (Table 2) and even though women who were HSV-2 positive on entry were significantly older than women who were HSV-2 negative on entry, age was not significant in combination with HSV-2 status on entry (Table 5). The experience of anal sex is common among these women and likely to be underreported, and this may explain the lack of significance of anal sex in this study. The presence or otherwise of herpetic ulcers, as observed by clinicians or self-reported, during each time interval could also have been recorded and may have helped to explain the apparent importance of recent HSV-2 infection as a risk factor for HIV-1 infection. The results could also be confounded by periods of high sexual activity during which the chance of being infected with HIV-1 and HSV-2 would both increase. However, only 2 women acquired both infections in the same time interval, suggesting that simultaneous infection, at least, is unlikely. Future studies should attempt to measure levels of sexual activity more precisely to separate behavioral effects on HIV-1 incidence from the biologic effects of HSV-2.

The key findings, from this and the other 3 studies discussed here, are as follows. First, prevalent or incident HSV-2 infection increases the incidence of HIV infection in men, but the range of estimates is large. The hazard ratio is 1.7 for prevalent infections in India[1]; the odds ratio is 17 for incident infections in Tanzania[17] (Fig. 3) and rather uncertain. Second, prevalent or incident HSV-2 infection does not increase the incidence of HIV infection in women significantly (when women who seroconverted during this and the

Tanzanian study[17] are compared with those who remained HSV-2 negative throughout) but does increase the incidence of HIV infection in women significantly by 6.0 (2.6–14.0) times when incident infection is analyzed as a time-dependent covariate in this study.

These studies, taken together, suggest that immediately after HSV-2 seroconversion, the risk of acquiring HIV-1 increases by up to 6 times in women and probably by rather more in men. As time goes by the effect appears to wane and may eventually disappear altogether in women, although it appears to remain significant in men. It is also likely that HIV-1 infection increases the risk of acquiring HSV-2 infection.[16] Here we have only considered the impact on HIV-1 infection of HSV-2 in the person being infected and not of HSV-2 infection in the person who is the source of the infection. Since people with HSV-2 secrete higher concentrations of HIV-1[3] and vice versa,[25] this may further enhance transmission of both viruses.

While the high prevalence of HSV-2 infection in many developing countries may be an important determinant of HIV infection, clinical manifestations of HSV-2 infection are diverse, individuals are often asymptomatic and may not seek medical care,[12] and many clinics do not test for HSV-2 infection routinely because they lack the facilities or fail to recognize the importance of the infection. Latent HSV-2 infections may reactivate and cause symptoms intermittently, rendering the carrier very contagious.[26]

It seems likely that HSV-2 is an important factor in determining the overall risk of HIV-1 infection, and the data reported here should help to guide the development of dynamical transmission models of HIV-1 epidemics that may help to reveal the extent and the importance of the synergy with HSV-2. Better data are still needed, although conducting such studies may now be more difficult with the advent of the widespread use of antiretroviral therapies. It is important that public health workers assess the extent to which symptomatic treatment of HSV-2 and, if it were available, a vaccine for HSV-2 may change the course of the HIV-1 epidemic.

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