

Risk Factors Influencing HIV Infection Incidence in a Rural African Population: A Nested Case-Control Study

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Background. Risk factors influencing the incidence of human immunodeficiency virus (HIV) infection were investigated in a case-control study nested within a community-randomized trial of treatment of syndromic sexually transmitted infections (STIs) in rural Tanzania.

Methods. Case patients were persons who became HIV positive, and control subjects were randomly selected from among persons who remained HIV negative. For each sex, we obtained adjusted odds ratios (ORs) and population-attributable fractions (PAFs) for biomedical and behavioral factors.

Results. We analyzed 92 case patients and 903 control subjects. In both sexes, the incidence of HIV infection was significantly higher in subjects with an HIV-positive spouse than in those with HIV-negative spouse (men: OR, 25.1; women: OR, 34.0). The incidence of HIV infection was significantly higher in those who became positive for herpes simplex virus type 2 (HSV-2) (men: OR, 5.60; women: OR, 4.76) and those who were HSV-2-positive at baseline (men: OR, 3.66; women: OR, 2.88) than in subjects who were HSV-2 negative. In women, living elsewhere (OR, 3.22) and never having given birth (OR, 4.27) were significant risk factors. After adjustment, the incidence of HIV infection was not significantly associated with a history of injections or STIs in either sex.

Conclusion. HSV-2 infection was the most important risk factor for HIV infection, which highlights the need for HSV-2 interventions in HIV infection control, and there were particularly strong associations with recent HSV-2 seroconversion. The PAF associated with having an HIV-positive spouse was low, but this is likely to increase during the epidemic.

HIV is, at present, the greatest threat to health in sub-Saharan Africa, with >3.1 million new cases in 2004 [1]. Despite the greater availability of antiretroviral therapy,

such therapy is costly and logistically complex, and effective HIV prevention remains an urgent priority. HIV prevention requires a clear understanding of the social, behavioral, and biomedical risk factors that influence the incidence of HIV infection, but few prospective studies in general populations in Africa have documented the relative importance of different factors.

Several studies have reported the aspects of sexual behavior that increase exposure to the virus, as well as biomedical cofactors that influence the risk of transmission per exposure. The incidence of HIV infection has been found to be higher in men and women in Uganda and Tanzania who reported greater numbers of sex partners [2–4]. In women in Tanzania and Rwanda, multiple sex partners and young age have been associated with a higher incidence of HIV infection [5, 6]. Several studies have demonstrated a higher incidence of HIV infection in men and women who are

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widowed, divorced, or separated [2, 4, 7, 8]. Studies in Uganda and Tanzania have shown a very high incidence of HIV infection among HIV-discordant married couples [9–11].

For biomedical cofactors, the evidence that other sexually transmitted infections (STIs) enhance HIV transmission has been reviewed extensively [12]. Longitudinal studies in Africa have demonstrated strong associations between ulcerative and non-ulcerative STIs and the incidence of HIV infection, and STIs are assumed to increase the infectiousness of HIV-positive subjects and the susceptibility of HIV-negative subjects [13, 14]. Herpes simplex virus type 2 (HSV-2) is one of the most common causes of genital ulcer disease (GUD), and its role in enhancing HIV transmission in Africa has been increasingly recognized [15–17]. There is also considerable evidence that male circumcision reduces the risk of acquiring HIV [2, 18–20].

Although sexual transmission is thought to account for most HIV infections in adults in Africa [1], parenteral transmission also needs to be considered. Worldwide, an estimated 5%–10% of HIV infections are caused by unscreened blood transfusions [21], and a further 2%–4% are caused by unsafe medical injections [22]. Some researchers have suggested that the latter may be an underestimate [23], although their conclusions have been challenged [24].

Data from a community-randomized trial in rural Mwanza have been reported elsewhere [25, 26], and the main results showed a decrease in the incidence of HIV infection after the implementation of treatment for syndromic STIs in interventional health-care units. Subsequent analysis showed that a substantial proportion of HIV infections in the control arm were attributable to GUD, particularly in men, with a much lower population-attributable fraction (PAF) in the intervention arm, which supports the hypothesis that the intervention acted by reducing the duration of symptomatic GUD [27]. An analysis of HSV-2 serologic data for a subset of trial participants demonstrated a strong association between the incidence of HIV infection and both prevalent and incident HSV-2 infection in men, with a weaker, nonsignificant association in women [15]. Further retrospective analysis showed a high incidence of HIV infection in the spouses of HIV-positive subjects in stable sex partnerships [11].

The present article brings together data on all these risk factors, which were measured in a case-control study nested within the community-randomized trial. The main objectives were to estimate the effect of each factor after adjustment for confounding factors and to estimate PAFs.

SUBJECTS, MATERIALS, AND METHODS

Between 1991 and 1994, a community-randomized trial was conducted in 12 rural communities in the Mwanza region of Tanzania, to measure the impact of improved STI treatment services delivered through government health-care units. The

design and results have been reported elsewhere [25, 26, 28]. Before the implementation of intervention, a cohort of ~1000 adults 15–54 years old were randomly selected from each of the communities. The communities were matched into 6 pairs, and 1 community from each pair was randomly selected to receive the intervention immediately; the other community received the intervention after the end of the trial. Two years later, cohort members were monitored to measure the effects of the intervention. At the follow-up survey, married subjects were asked to identify their spouse if he or she lived in the same community.

At the baseline and follow-up surveys, samples of venous blood were obtained from consenting subjects, and these were tested for HIV antibodies by use of standard protocols [25]. After the final survey, an unmatched case-control study was undertaken to examine risk factors influencing the incidence of HIV infection. Case patients were defined as persons who became HIV positive, and control subjects were chosen as a 1-in-8 random sample of subjects who remained persistently HIV negative. With ~100 case patients and 1000 control subjects, the study had 80% power, in each sex, to detect an odds ratio (OR) of 2.5 for risk factors present in 30% of the population.

The case-control study took place from August 1994 to February 1995, 3–6 months after the follow-up survey. Informed consent was obtained, and a structured questionnaire was administered to subjects to ascertain sociodemographic characteristics and detailed information on sexual behavior. Non-medical personnel were trained in interview techniques and were unaware of the HIV status of participants. Stable partnerships were identified at follow-up, and the HIV status of each partner was obtained from the baseline data [11]. In 2003, stored aliquots of serum from the baseline and follow-up surveys were identified for all participants in the case-control study; these were tested for HSV-2 antibodies by use of a commercial ELISA (HSV2 IgG; Kalon Bio).

Sociodemographic and biomedical data from the follow-up survey and behavioral data from the case-control survey were used, with separate analyses for men and women. Logistic regression was used to obtain ORs for sociodemographic, biomedical, and behavioral factors, using a proximate-determinants conceptual framework [29]. Sociodemographic factors were adjusted for age group (15–19, 20–34, or 35–54 years), residence stratum (low- or high-risk community), and treatment arm, and biomedical and behavioral factors were additionally adjusted for other significant factors ($P < .05$). Significance was assessed using the likelihood ratio test. Dbase software (version 4; Borland) was used for data entry, and Stata software (version 8.0; Stata) was used for the analysis. The PAF of incidence of HIV infection associated with significant risk factors was estimated using the formula $PAF = p_c(RR - 1)/RR$, where p_c is the proportion of case patients exposed and RR

was estimated by the adjusted OR [30]. Confidence intervals for the PAF were obtained using the *aflogit* command in Stata [31]. Ethics clearance for the study was given by the Tanzanian Commission of Science and Technology and the ethics committee of the London School of Hygiene and Tropical Medicine.

RESULTS

After the follow-up survey, all 130 case patients with incident HIV infection and 1157 HIV-negative control subjects were selected for the case-control study. Data were successfully collected from 97 (75%) case patients and 948 (82%) control subjects (table 1). After the exclusion of 50 subjects for whom there was some doubt concerning their identity, 92 case patients and 903 control subjects were included in the analysis.

Sociodemographic risk factors. The lowest incidence was observed in men <20 years old and in women ≥ 35 years old, although differences by age were not significant (table 2). In both sexes, there was no significant association between the incidence of HIV infection and occupation, travel to Mwanza City, ethnic group, or religion. In men, a higher level of education was associated with a significantly lower risk of incidence of HIV infection, although this was not evident in women. In both sexes, there was an increased risk of HIV infection in those who had lived in a different community at some time during the preceding 2 years, and this reached statistical significance in women (OR, 3.79 [95% confidence interval {CI}, 1.82–7.90]). In both sexes, a higher incidence of HIV infection was seen in those who were divorced or widowed, and in men this reached statistical significance. In women, there was a significant association between the incidence of HIV infection and never having given birth, which persisted after adjustment for marital status (OR, 4.11 [95% CI, 1.47–11.5]).

After adjustment for other significant factors, including behavioral and biomedical factors, none of the sociodemographic factors remained significant in men, but living in a different community (OR, 3.21 [95% CI, 1.36–7.60]) and never having given birth (OR, 4.27 [95% CI, 1.40–13.0]) remained significant in women.

Behavioral and biomedical risk factors in men. In men, the association between the incidence of HIV infection and behavioral and biomedical risk factors is shown in table 3, after adjustment for age, residence stratum, treatment arm, and other factors that remained significant in the multivariate analysis.

The HIV status of a spouse was available for 235 (61%) of 384 men who were currently married. There was a very high risk of infection for men married to an HIV-positive spouse (OR, 25.1 [95% CI, 3.37–187]), compared with that for men married to an HIV-negative spouse, giving an estimated PAF of 8.0% (95% CI, –5.5% to 19.9%). There was no significant association between male circumcision and HIV infection.

During the 2-year follow-up period, 34% of male control sub-

Table 1. Subjects selected for nested case-control study, those participating, and those analyzed.

Group	Male		Female	
	Case patients ^a	Control subjects ^b	Case patients ^a	Control subjects ^b
Selected	71	568	59	589
Participated	52	469	45	479
Participation rate, %	73	83	76	81
Excluded from analysis ^c	2	24	3	21
Total analyzed	50	445	42	458

NOTE. Data are no. of subjects, unless otherwise indicated.

^a HIV negative at baseline and HIV positive at follow-up, after 2 years.

^b HIV negative at baseline and follow-up.

^c Excluded because of doubts concerning the identity of the subject seen at the case-control interview.

jects reported having received injections in a hospital or health-care center only, and a further 6% reported having received injections elsewhere. After adjustment for other risk factors, there was a nonsignificant higher risk of HIV infection in men who received injections in a health-care facility (OR, 1.94 [95% CI, 0.84–4.49]) or elsewhere (OR, 2.47 [95% CI, 0.71–8.64]).

At least 1 syndromic STI during the preceding 2 years was reported by 52% of male case patients and 29% of male control subjects. After adjustment for age, residence stratum, and treatment arm, there was a highly significant association between the incidence of HIV infection and reported STI (OR, 2.66 [95% CI, 1.41–5.00]), the strongest association being with genital ulcer syndrome (GUS) (OR, 3.08 [95% CI, 1.59–6.00]). After additional adjustment for the HIV status of the spouse and the HSV-2 status of the subject, these associations were no longer significant (table 3). Because a reported STI and HSV-2 status were strongly correlated, the analysis was repeated without adjustment for HSV-2 status. The incidence of HIV infection was significantly associated with any reported STI (OR, 2.32 [95% CI, 1.19–4.51]), GUS (OR, 2.71 [95% CI, 1.32–5.54]), and genital discharge syndrome (OR, 2.11 [95% CI, 1.04–4.28]). The estimated PAF for GUS from this analysis was 24.0% (95% CI, 2.8%–40.5%).

Men commonly reported having had multiple sex partners; 79% of control subjects reported at least 5 lifetime partners, and 65% reported ≥ 2 partners during the preceding 2 years. There was no significant association between the incidence of HIV infection and the reported number of partners, although, after adjustment for other risk factors (including HSV-2 status), the OR for men reporting ≥ 5 lifetime sex partners was 4.94 ($P = .13$ [95% CI, 0.63–39.0]), compared with those who reported <5 partners. There was no significant association between the incidence of HIV infection and reported condom use, casual sex partners, or age at first intercourse. No case patients and 16 control subjects reported having had no sex partner in the preceding 2 years.

Table 2. Risk factors influencing the incidence of HIV infection: odds ratios (ORs) for selected sociodemographic factors, by sex.

Variable	Male				Female			
	Case patients	Control subjects	Adjusted OR ^a (95% CI)	P	Case patients	Control subjects	Adjusted OR ^a (95% CI)	P
Age at baseline, years								
15–19	3	63	1	.12	7	66	1	.18
20–34	24	201	3.11 (0.89–10.9)		25	218	1.03 (0.42–2.52)	
35–54	23	181	2.90 (0.79–10.2)		10	174	0.53 (0.19–1.46)	
Residence stratum								
Low risk	21	323	1	<.001	23	306	1	.11
High risk	29	122	3.93 (2.14–7.24)		19	152	1.69 (0.89–3.23)	
Treatment arm								
Comparison	32	247	1	.2	29	233	1	.025
Intervention	18	198	0.67 (0.36–1.26)		13	225	0.47 (0.24–0.93)	
Education								
None/adult	15	73	1	.03	15	215	1	.7
Primary/above	35	371	0.46 (0.22–0.94)		27	243	1.15 (0.55–2.38)	
Occupation								
Farming	26	220	1	.6	31	379	1	.4
Other	24	225	0.87 (0.47–1.62)		11	79	1.37 (0.64–2.93)	
Lived elsewhere during the preceding 2 years								
No	40	389	1	.07	27	410	1	<.001
Yes	10	56	2.15 (0.96–4.79)		15	47	3.79 (1.82–7.90)	
Traveled to Mwanza City within the preceding year								
No	28	258	1	.13	32	369	1	.9
Yes	22	187	0.60 (0.31–1.17)		10	89	0.96 (0.43–2.13)	
Ethnic group								
Sukuma	44	310	1	.1	34	309	1	.25
Other	6	135	0.47 (0.19–1.18)		8	149	0.61 (0.27–1.41)	
Religion								
Catholic	21	172	1	.7	17	197	1	.6
Protestant	17	127	1.07 (0.53–2.17)		16	127	1.44 (0.69–2.97)	
Other	12	146	0.76 (0.35–1.64)		9	134	1.00 (0.42–2.35)	
Current marital status								
Married	34	350	1	.02	28	359	1	.26
Widowed/divorced	9	21	3.65 (1.49–8.92)		9	63	1.98 (0.85–4.63)	
Never married	7	74	1.56 (0.52–4.65)		5	36	1.57 (0.49–5.05)	
Ever given birth								
Yes		32	421	...	<.001
No		10	37	4.92 (1.86–13.0)	

NOTE. Data are no. of subjects, unless otherwise indicated. CI, confidence interval.

^a ORs adjusted for age, residence stratum, and treatment arm. P values were derived from the likelihood-ratio test for differences in odds between categories.

Data on HSV-2 status were missing or indeterminate for 39 men. After adjustment for the HIV status of a spouse, the incidence of HIV infection was significantly higher in men who became HSV-2 positive during the 2-year follow-up period (OR, 5.60 [95% CI, 1.67–18.80]) and in men who were HSV-2 positive at baseline (OR, 3.66 [95% CI, 1.28–10.40]) than in those who remained HSV-2 negative. The overall PAF for HSV-2 infection was 64.9% (95% CI, 18.2%–85.0%): 20.5% for those who became HSV-2 positive and 44.4% for those with prevalent HSV-2 infection. There was no significant association between the incidence of HIV infection and testing positive for syphilis.

Behavioral and biomedical risk factors in women. In women, the association between the incidence of HIV infection

and behavioral and biomedical risk factors, after adjustment for age, residence stratum, treatment arm, and other factors that remained significant in the multivariate analysis is shown in table 4. The HIV status of a spouse was available for 209 (54%) of 387 women who were currently married. There was a very high risk of infection in women married to an HIV-positive spouse (OR, 34.0 [95% CI, 5.77–200]), compared with women married to an HIV-negative spouse, giving an estimated PAF of 13.1% (95% CI, –1.3% to 25.5%).

During the 2-year follow-up period, 47% of female control subjects reported having received injections in a hospital or health-care center only, and a further 8% reported having received injections elsewhere. After adjustment for other risk fac-

Table 3. Risk factors influencing the incidence of HIV infection: odds ratios (ORs) for selected behavioral and biomedical factors in men.

Variables	Case patients, no.	Control subjects, no.	Adjusted OR ^a (95% CI)	P ^a	Adjusted OR ^b (95% CI)	P ^b
HIV status of marital partner						
Negative	10	216	1	<.001	1	<.001
Positive	6	3	56.3 (10.9–290)		25.1 (3.37–187)	
None/not known	34	226	4.06 (1.87–8.80)		4.21 (1.69–10.5)	
Circumcised						
No	34	329	1	.5	1	.3
Yes	16	111	1.27 (0.66–2.44)		1.57 (0.70–3.51)	
Injections during the preceding 2 years						
None	23	265	1	.3	1	.18
Hospital/clinic	22	153	1.49 (0.79–2.82)		1.94 (0.84–4.49)	
Elsewhere	5	26	2.08 (0.70–6.19)		2.47 (0.71–8.64)	
STI during the preceding 2 years ^c						
No	24	316	1	.002	1	.12
Yes	26	129	2.66 (1.41–5.00)		1.87 (0.86–4.07)	
Genital ulcer syndrome during the preceding 2 years ^c						
No	31	365	1	<.001	1	.3
Yes	19	80	3.08 (1.59–6.00)		1.62 (0.68–3.87)	
Genital discharge syndrome during the preceding 2 years ^c						
No	31	356	1	.008	1	.12
Yes	19	89	2.50 (1.29–4.85)		1.98 (0.86–4.58)	
Sex partners ever, no.						
0–4	4	74	1 ^d	.7	1 ^d	.5
5–9	11	79	1.73 (0.51–5.92)		7.22 (0.83–62.7)	
≥10	35	292	1.44 (0.47–4.42)		4.29 (0.54–34.3)	
Sex partners during the preceding year, no.						
0 or 1	28	211	1	.3	1	.6
≥2	22	233	0.72 (0.39–1.34)		0.81 (0.37–1.76)	
Sex partners during the preceding 2 years, no.						
0 or 1	20	157	1	.4	1	.9
≥2	30	288	0.78 (0.42–1.45)		1.05 (0.47–2.34)	
Casual sex partners during the preceding year						
No	25	216	1	.8	1	.9
Yes	25	229	0.95 (0.51–1.77)		0.94 (0.43–2.04)	
Ever used condom						
No	43	373	1	.8	1	.9
Yes	7	72	0.93 (0.38–2.26)		1.00 (0.33–3.04)	
Age at first intercourse, years ^e						
<15	11	110	1 ^d	.6	1 ^d	.9
15–19	32	278	1.14 (0.54–2.39)		0.91 (0.38–2.16)	
≥20	5	32	1.35 (0.42–4.34)		1.12 (0.29–4.35)	
Syphilis serologic results ^f						
Negative	38	355	1	.9	1	.9
Positive at baseline	10	73	0.86 (0.40–1.88)		0.96 (0.39–2.40)	
Became positive	2	17	0.86 (0.18–4.07)		1.40 (0.27–7.22)	
HSV-2 serologic results						
Not tested	14	25	
Negative	5	184	1 ^g	<.001	1 ^g	.003
Positive at baseline	22	193	3.89 (1.39–10.9)		3.66 (1.28–10.4)	
Became positive	9	43	6.64 (2.04–21.6)		5.60 (1.67–18.8)	

NOTE. CI, confidence interval; HSV, herpes simplex virus; STI, sexually transmitted infection.

^a Adjusted for age, residence stratum, and treatment arm. *P* values were derived from the likelihood-ratio test (LRT) for differences in odds between categories.

^b Adjusted for age, residence stratum, treatment arm, HIV status of marital partner, and HSV-2 serologic results. *P* values were derived from the LRT for differences in odds between categories.

^c Coded as “yes” if the presence of an STI was reported at either follow-up or on the case-control questionnaire.

^d Test for trend.

^e Seven men who reported never having had sex were excluded.

^f Syphilis status defined by *Treponema pallidum* hemagglutination assay at baseline and follow-up.

^g Test for trend (excluding those not tested): negative, baseline positive, or became positive.

tors, the incidence of HIV infection was slightly higher in women who received injections in a health-care facility (OR, 1.48 [95% CI, 0.63–3.48]), but there was a higher risk of HIV infection in women who received injections elsewhere (OR, 3.63 [95% CI, 1.07–12.40]), although overall this association was not statistically significant.

Syndromic STIs were less common in women than men, with 24% of female case patients and 19% of female control subjects reporting at least 1 STI during the preceding 2 years. No significant associations were observed between the incidence of HIV infection and reported syndromic STIs.

Multiple sex partners were reported less frequently by women than men, with 43% of female control subjects reporting ≥ 5 lifetime partners and 20% reporting ≥ 2 partners during the preceding 2 years. After adjustment for age, residence stratum, and treatment arm, the incidence of HIV infection was significantly higher in women reporting ≥ 2 partners during the preceding year (OR, 2.46 [95% CI, 1.13–5.34]) or 2 years (OR, 2.19 [95% CI, 1.10–4.38]) or a casual sex partner during the preceding year (OR, 2.41 [95% CI, 1.21–4.78]), although these associations were not significant after adjustment for other risk factors. There was no significant association between the incidence of HIV infection and reported condom use or age at first intercourse.

Data on HSV-2 status were missing or indeterminate for 33 women. After adjustment for other risk factors, the incidence of HIV infection was significantly higher in women who became HSV-2 positive during the 2-year follow-up period (OR, 4.76 [95% CI, 1.21–18.80]) and in women who were HSV-2 positive at baseline (OR, 2.88 [95% CI, 0.92–8.96]) than in women who remained HSV-2 negative. The overall PAF for HSV-2 infection was 58.7% (95% CI, –2.5% to 83.3%): 12.8% for those who became HSV-2 positive and 45.9% for those with prevalent HSV-2 infection. There was no significant association between the incidence of HIV infection and testing positive for syphilis.

DISCUSSION

The present article reports on risk factors for HIV infection in a rural population at a time when the incidence of HIV infection was <1% per year and the prevalence of HIV was rising. The case-control study was nested within a community-randomized trial [25], and the trial cohort was randomly selected from 12 rural communities, so our findings should give an accurate picture of the contribution of different risk factors to the incidence of HIV infection. However, there were some losses to follow-up during the trial, and not all selected subjects were successfully interviewed for the case-control study, so we cannot exclude the possibility of selection bias. Underrepresentation of mobile members of the community should not have compromised the internal validity of the study, but it may have resulted in an underestimation of the PAFs of some risk factors

if these were more common in the missing subjects. Diagnostic error and recall bias are likely to have been nondifferential, diluting associations, and to have further reduced estimated PAFs. A further limitation of the study is its focus on individual-level risk factors for HIV acquisition, such that the full contribution of some risk factors to HIV transmission at the population level may not have been captured.

One strength of the study is the availability of data on a wide range of potential confounding factors. Previous articles have reported results on specific risk factors from the present study, but we have extended those analyses by considering the joint effects of all potential risk factors [11, 15, 25, 27]. To avoid excessive parameterization of the logistic-regression model, given the relatively small number of cases, associations were adjusted only for risk factors that remained significant in the multivariate analysis. After adjustment for age, residence stratum, and treatment arm, there was little evidence of between-community variation, so finer adjustment for community of residence was unnecessary.

The present results confirm our previous finding that having an HIV-positive spouse puts an individual at very high risk for HIV infection [11]. In the present study, we analyzed a smaller number of HIV-discordant partners, all of whom had responded to the case-control questionnaire as incident case patients or selected control subjects. We also adjusted these associations for other risk factors, including HSV-2 status, which somewhat reduced the strength of the association in men. We estimate that 11% of incident HIV infections were attributable to being married to an HIV-positive spouse, which is consistent with the relatively early stages of an HIV epidemic, when most new infections occur outside of marriage. Although this is relatively low, as the epidemic progresses, more discordant partnerships may be seen, and a greater number of new infections will be attributable to transmission within stable partnerships [9, 10]. This emphasizes the increasing importance in mature HIV epidemics of interventions targeted at stable partnerships, such as voluntary counseling and testing services for couples or effective vaginal microbicides.

The most important risk factor influencing the incidence of HIV infection identified in this population was testing positive for HSV-2, which showed estimated PAFs of 65% in men and 59% in women. These results differ somewhat from those reported previously, which showed a strong association in men but a weaker and nonsignificant association in women [15]. There were several differences between the 2 studies that may explain these observations. First, the previous study was based on the same case patients but a subset of control subjects that was heavily weighted toward the youngest age groups, whereas the present study assayed the HSV-2 status of all study subjects. Because the observed effect of HSV-2 status in women was stronger at older ages, a greater overall effect was seen in the present

Table 4. Risk factors influencing the incidence of HIV infection: odds ratios (ORs) for selected behavioral and biomedical factors in women.

Variable	Case patients, no.	Control subjects, no.	Adjusted OR ^a (95% CI)	P ^a	Adjusted OR ^b (95% CI)	P ^b
HIV status of marital partner						
Negative	4	194	1	<.001	1	<.001
Positive	5	6	30.8 (6.27–151)		34.0 (5.77–200)	
None/not known	33	258	6.08 (2.08–17.5)		5.13 (1.47–17.9)	
Injections during the preceding 2 years						
None	16	214	1	.4	1	.14
Hospital/clinic	20	206	1.29 (0.64–2.57)		1.48 (0.63–3.48)	
Elsewhere	6	36	2.10 (0.75–5.85)		3.63 (1.07–12.4)	
STI during the preceding 2 years ^c						
No	32	370	1	.6	1	.9
Yes	10	88	1.22 (0.57–2.61)		0.93 (0.39–2.21)	
Genital ulcer syndrome during the preceding 2 years ^c						
No	37	410	1	.9	1	.9
Yes	5	48	1.09 (0.40–2.95)		1.09 (0.36–3.26)	
Genital discharge syndrome during the preceding 2 years ^c						
No	36	390	1	.9	1	.4
Yes	6	68	0.92 (0.37–2.30)		0.67 (0.24–1.84)	
Sex partners ever, no.						
0 or 1	2	61	1 ^d	.06	1 ^d	.3
2–4	16	199	2.30 (0.51–10.4)		1.21 (0.23–6.26)	
≥5	24	198	3.64 (0.81–16.4)		1.77 (0.34–9.27)	
Sex partners during the preceding year, no.						
0 or 1	31	404	1	.02	1	.3
≥2	11	51	2.46 (1.13–5.34)		1.61 (0.65–3.98)	
Sex partners during the preceding 2 years, no.						
0 or 1	26	367		.03	1	.7
≥2	16	91	2.19 (1.10–4.38)		1.16 (0.50–2.66)	
Casual sex partner during the preceding year						
No	25	362	1	.01	1	.9
Yes	17	91	2.41 (1.21–4.78)		1.03 (0.44–2.45)	
Ever used condom						
No	39	439	1	.4	1	.4
Yes	3	19	1.83 (0.51–6.60)		1.72 (0.42–7.07)	
Age at first intercourse, years ^e						
<15	11	122	1 ^d	.9	1 ^d	.9
15–19	28	264	1.20 (0.57–2.52)		1.30 (0.52–3.23)	
≥20	1	29	0.42 (0.05–3.45)		0.68 (0.08–6.04)	
Syphilis serologic results ^f						
Negative	29	365	1	.2	1	.6
Positive at baseline	8	71	1.41 (0.61–3.28)		0.67 (0.23–1.95)	
Became positive	5	22	2.76 (0.94–8.09)		1.53 (0.40–5.81)	
HSV-2 serologic results						
Not tested	5	28	
Negative	5	129	1 ^g	.006	1 ^g	.02
Positive at baseline	26	273	2.98 (1.05–8.44)		2.88 (0.92–8.96)	
Became positive	6	28	5.49 (1.53–19.8)		4.76 (1.21–18.8)	

NOTE. Data are no. of subjects, unless otherwise indicated. CI, confidence interval; HSV, herpes simplex virus; STI, sexually transmitted infection.

^a Adjusted for age, residence stratum, and treatment arm. *P* values were derived from the likelihood-ratio test (LRT) for differences in odds between categories.

^b Adjusted for age, residence stratum, treatment arm, living away from community within the preceding 2 years, ever having given birth, HIV status of marital partner, and HSV-2 serologic results. *P* values were derived from the LRT for differences in odds between categories.

^c Coded as “yes” if the presence of an STI was reported at either follow-up or on the case-control questionnaire.

^d Test for trend.

^e Five women who reported never having had sex were excluded.

^f Syphilis status defined by *Treponema pallidum* hemagglutination assay at baseline and follow-up.

^g Test for trend (excluding those not tested): negative, baseline positive, or became positive.

study. Second, the present study was restricted to subjects with complete data on behavioral variables, so that a fully adjusted analysis could be performed. Because HIV and HSV-2 share a common mode of transmission, their association may be confounded by common risk factors [32], although adjustment for sociodemographic and behavioral factors in the present study did not substantially change the observed association. Third, serum samples in the previous study were analyzed using an in-house monoclonal blocking assay [15, 33], whereas the present study used a commercially available ELISA; however, both assays have high sensitivity and specificity [33, 34], so it seems unlikely that this would account for the different findings.

A recent meta-analysis concluded that, on the basis of published longitudinal studies, prevalent HSV-2 infection doubled the risk of acquiring HIV [35]. However, only 1 of these studies, in Thai sex workers, was conducted in women, and it paradoxically showed a lower incidence of HIV infection in HSV-2-positive women [36]. It is clearly important to collect more data on this association in women, given that our findings are particularly significant in showing a strong and statistically significant effect in women, in contrast to the Thai findings and consistent with previous results in men. We found a stronger association with HSV-2 positivity than with prevalent HSV-2 infection, which may reflect the higher frequency and severity of herpetic ulcers during the early stages of HSV-2 infection [37]. The hypothesis that genital herpes increases the risk of both acquisition and transmission of HIV has led to intervention trials to assess the impact of herpes treatment as a control measure for HIV infection.

Previous studies have shown that HIV transmission is enhanced by STIs other than herpes [5, 13]. After adjustment for confounding factors other than HSV-2, men in the present study who reported a syndromic STI were at a significantly higher risk for HIV infection (OR, 2.32). Some of the reported syndromic STIs, particularly GUS, would have been due to herpes, so it is not surprising that further adjustment for HSV-2 status reduced the strength of the association. Reported syndromic STIs in community-based studies have been known to lack sensitivity and specificity as markers of laboratory diagnosed STIs, especially in women [38]. This may explain the lack of an association in women.

In common with other studies, our results show a higher incidence of HIV infection in those who are divorced or widowed [2, 4, 7] or who have lived elsewhere [8], possibly because these subgroups are more likely to have had multiple sex partners. As in data from Uganda, women who reported multiple sex partners were at a greater risk for HIV infection [2, 3], although, unlike Uganda, there was no such association in men [2]. The misreporting of sexual behavior continues to complicate the assessment of the association between HIV infection and the number of sex partners in both men and women.

A meta-analysis based on previous studies showed reasonably consistent evidence that male circumcision provides some protection against HIV acquisition [18], and the lack of such an effect in the present study was therefore surprising. In the Mwanza region, circumcision is more common in geographical and social strata that are otherwise at a higher risk for HIV infection. However, we cannot explain why the association in our study was the opposite of that expected, even after adjustment for such factors.

The present article reports a nonsignificant association between injections and the incidence of HIV infection in both sexes. The strongest association was with injections given in places other than formal health-care facilities, where unsterilized equipment may be used, although few subjects reported such injections. Results from a case-control study in Uganda also showed associations between injections and the incidence of HIV infection [2], whereas other studies showed no association [3, 6]. Gisselquist and Potterat [23] argued that injections may be a more important cause of HIV transmission than multiple sex partners. Reverse causation and confounding may partly explain this relationship, because injections may be reported more frequently for the treatment of illnesses associated with primary HIV infection or for STIs responsible for enhancing the transmission of HIV. Although we acknowledge the need to eliminate unsafe injections, the strongest associations observed in the present study were with markers of sexual activity, which supports the view that the sexual transmission of HIV predominates in this population and is exacerbated by the high prevalence of STIs, including HSV-2 infection [24].

In conclusion, our results show that risk factors for HIV infection in this rural population are complex and interlinked. As the epidemic matures and HIV spreads further into the general population, a greater proportion of people will be infected by their regular partner, which implies an increasing need for interventions targeted at stable partnerships. The treatment of syndromic STIs has been shown to reduce the incidence of HIV infection in populations with high rates of curable STIs. Herpes is the most common incurable STI; in the present study, it was responsible for the largest PAF of new HIV infections. A major focus of future research must be to develop and evaluate interventions to reduce the incidence and clinical expression of HSV-2 infection and to assess their impact as HIV infection control measures.

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