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Incident HIV and herpes simplex virus type 2 infection among men in Rakai, Uganda

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Abstract

Objective—Herpes simplex virus type 2 (HSV-2) infection is associated with an increased risk for acquiring HIV, but little is known about the temporal sequence of these infections.

Design—Six thousand three hundred ninety-six men were evaluated for serologic HSV-2 and HIV infections and behaviors during a male circumcision trial in Rakai, Uganda.

Methods—HIV and HSV-2 status were determined using enzyme-linked immunosorbent assays and confirmed by HIV-1 and HSV-2 western blots. A Poisson multivariable model was used to estimate adjusted incidence rate ratios of HIV acquisition associated with HSV-2 and other covariates.

Results—HIV incidence was 1.09/100 person-years and acquisition was associated with incident HSV-2 infection [adjusted incidence rate ratio (adjIRR) 5.28, 95% confidence interval (CI) 2.79–9.98], chronic HSV-2 infection (adjIRR 2.78, 95% CI 1.64–5.68), genital ulcer disease, urethral discharge, genital washing after intercourse, being unmarried, and being uncircumcised. Sixteen men acquired both HIV and HSV-2 during the trial: four acquired HIV first, three acquired HSV-2 first, and nine acquired both infections in the same follow-up interval.

Conclusion—The findings suggest that unsafe sex places men at risk of both HIV and HSV-2 infections, and it is unclear whether HSV-2 acquisition is a cofactor for HIV infection or a marker of correlated sexual exposures. This reinforces the need for promotion of safe sex as the primary method of prevention of both viruses.

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There are no conflicts of interest.

Keywords

herpes simplex virus type 2; HIV; male circumcision; risk factors; Uganda

Introduction

Herpes simplex virus type 2 (HSV-2) infection is associated with an approximately three-fold increased risk for acquiring HIV in observational studies [1–5], and the risk for HIV infection appears to be higher with incident HSV-2 [1,6]. Although HIV and HSV-2 are associated, it is unknown whether one virus increases the likelihood of infection with the other virus (i.e., biologic cofactor effect) or whether the association is due to confounding by high-risk sexual behaviors that increase the risk of both these sexually transmitted infections (STIs). HSV-2 is a marker of high-risk sexual behavior [7–9]. In addition, as HSV-2 is more infectious than HIV, it likely that herpes infection will precede HIV infection, so one cannot necessarily infer causality from the temporal sequence of infections. It is biologically plausible that HSV-2 is a biologic cofactor for HIV acquisition, as HSV-2 causes ulcers breaching the mucosal barrier with recruitment of dendritic cells and CD4+ T cells expressing CCR5 into areas of HSV-2 replication in clinical and subclinical mucosal lesions [3,10,11]. However, two trials of HSV suppression with acyclovir in HSV-2-positive/HIV-negative participants failed to show reductions in HIV acquisition [12,13], suggesting that serologic evidence of HSV-2 infection may not, in itself, be a risk factor for HIV acquisition.

Here we report risk factors for incident HIV and the temporal sequence of dual HSV-2/HIV infections in men enrolled in randomized trials of male circumcision in Rakai, Uganda.

Patients and methods

We evaluated HIV and HSV-2 acquisition in 6396 men aged 15–49 years enrolled in two trials of male circumcision for HIV and STI prevention in Rakai district, Uganda, as previously reported [14–16]. Men who had contraindications for surgery (e.g., anemia, active genital infection) were treated, and if their medical condition resolved, they were re-screened and were enrolled in the trial. Those with anatomical abnormalities (e.g., hypospadias) or medical indications for surgery (e.g., severe phimosis) were excluded. Participants provided written informed consent prior to screening and at enrollment. Men were randomly assigned to receive immediate circumcision or circumcision delayed for 24 months. Serologic testing (HIV, HSV-2, and syphilis), physical examinations, and interviews to ascertain sociodemographic characteristics and sexual risk behaviors were conducted at baseline and repeated at 6, 12, and 24 months follow-up. Serum was stored at −70°C. All participants were offered free HIV counseling and testing, health education, and condoms at each visit. All participants found to be HIV-positive were referred for free care to the Rakai Health Sciences Program HIV care and treatment services funded by the President’s Emergency Plan for AIDS Relief.

The trials were approved by the HIV Subcommittee of the Ugandan National Council for Research and Technology (Kampala, Uganda) and by three institutional review boards: the Science and Ethics Committee of the Uganda Virus Research Institute (Entebbe, Uganda), the Committee for Human Research at Johns Hopkins University Bloomberg School of Public Health (Baltimore, Maryland, USA), and the Western Institutional Review Board (Olympia, Washington, USA). The trials were overseen by independent Data Safety Monitoring Boards [14,15] and were registered with Clinical.Trials.Gov numbers NCT00425984 and NCT00124878.

HSV-2, HIV, and syphilis detection

HSV-2 infection was determined by HSV-2 enzyme-linked immunosorbent assay (ELISA) (Kalon Biological Ltd, Guilford, UK), as previously described [9,15]. An HSV-2 seroconversion was defined as a negative enrollment serology followed by a positive follow-up serology with confirmation by Euroimmun western blot (Euroimmun, Lubeck, Germany). As there is a time lag from primary infection to seroconversion using the Kalon HSV-2 assay [17], all dual HIV/HSV-2 seroconverters were assessed by University of Washington Western blot (UWWB), which detects HSV-2 seroconversion within 4 weeks from HSV-2 infection when a panel of subsequent samples are evaluated [18]. Atypical and indeterminate UWWB results were considered positive to ensure the shortest time interval from HSV-2 infection to antibody detection.

HIV status was determined using two separate ELISAs and confirmed by HIV-1 western blot, as previously described [14]. Active *Treponema pallidum* infection was determined by a positive rapid plasma reagin (RPR) followed by a positive *T. pallidum* particle agglutination assay (TPPA), as previously described [15].

Statistical analysis

For HIV and HSV-2 incidence rate and person-time calculations, it was assumed that infection occurred at the mid-time point between the last negative and first positive serological tests. Time from enrollment was accumulated for the 24-month follow-up visit or to the last visit with an available sample, and HIV incidence was estimated per 100 person-years.

For incident HIV analyses, associations with fixed covariates such as age, marital status, and education at enrollment, and by time-varying covariates such as sexual risk behaviors (e.g., number of partners, nonmarital relationships, condom use, and alcohol use with sex) reported during follow-up visits were assessed by Poisson regression. Risk factors with a *P* value less than 0.15 in univariate analysis were entered into a Poisson multivariable model to estimate adjusted incidence rate ratios (adjIRRs) and 95% confidence intervals (95% CIs) of HIV acquisition associated with fixed and time-varying covariates. Only sexually active individuals were included in the adjusted analysis.

Results

During the 2-year follow-up of initially HIV-uninfected men, the HIV incidence rate was 1.09/100 person-years (105/9604 person-years) (Table 1). In multivariate Poisson regression, the adjIRRs of HIV acquisition were significantly increased with enrollment HSV-2 prevalent positive serostatus (adjIRR 2.78, 95% CI 1.64–5.68), baseline indeterminate HSV-2 serostatus (adjIRR 2.99, 95% CI 1.58–5.68), and markedly increased with HSV-2 seroconversion during the trial (adjIRR 5.28, 95% CI 2.79–9.98). HIV risk was also increased with washing genitals after sexual intercourse, and self-reported GUD and urethral discharge symptoms. HIV acquisition was decreased with current marriage and male circumcision.

As HSV-2 infection often causes symptomatic GUD, we conducted a sensitivity analysis excluding GUD symptoms from the multivariate analysis. When GUD was excluded from the adjusted analysis, the risks of HIV acquisition among HSV-2-prevalent positive individuals was adjIRR 2.90 (95% CI 1.71–4.92) and among HSV-2 seroconverters was adjIRR 6.00 (95% CI 3.19–11.24). These estimates are higher than the adjusted risks of HIV seroconversion, which controlled for GUD (Table 1), suggesting that symptomatic ulceration may be in the causal pathway between serologic HSV-2 and HIV acquisition. To evaluate the role of confounding due to sexual risk behaviors, we also conducted sensitivity analyses that adjusted for baseline demographic characteristics, but excluded sexual risk behaviors and GUD

symptoms. The risk of HIV acquisition was similar to the fully adjusted analysis for the HSV-2-prevalent positive individuals (adjIRR 3.04, 95% CI 1.84–5.03) and HSV-2 seroconverters (adjIRR 5.63, 95% CI 3.05–10.41).

As HSV-2 seroconversion was most strongly associated with HIV acquisition during the trial, the timing of HIV and HSV-2 infection was determined for 17 individuals who acquired both HIV and HSV-2 during the 2-year follow-up. One participant did not have month 6 or year 1 samples available for evaluation of the timing of co-infections. Of the 16 dual HIV, HSV-2 seroconverter men for whom timing of both infections could be assessed, four acquired HIV in follow-up intervals prior to HSV-2 (25.0%), three acquired HSV-2 prior to HIV (18.8%), and nine (56.3%) acquired HIV and HSV-2 in the same follow-up interval (Table 2).

To determine whether acute HSV-2 infection is associated with HIV acquisition, multivariate analysis that incorporated the temporal sequence data was performed. The four individuals who acquired HIV prior to acquiring HSV-2 were considered persistent HSV-2-negative individuals, as they acquired HIV first. If it is assumed that all nine individuals with dual infections in the same follow-up interval acquired HSV-2 prior to HIV, the HIV acquisition rate was higher among incident HSV-2 individuals than among persistent negative individuals (adjIRR 3.40, 95% CI 1.74–6.67). If it is assumed that only the three individuals with documented incident HSV-2 prior to HIV truly acquired HSV-2 before HIV (i.e., the nine men with simultaneous HIV/HSV-2 acquisition were excluded), incident HSV-2 individuals had a similar rate of HIV acquisition compared with the persistent negative individuals (adjIRR 1.06, 95% CI 0.37–3.06).

Discussion

The findings in this study are similar to those of previous studies that have documented that HSV-2 seroconversion is associated with HIV acquisition [6,19,20]. However, previous studies did not evaluate the timing of dual HIV/HSV-2 seroconversion using sensitive assays that can detect antibodies to both HIV and HSV-2 within approximately 4 weeks of infection. In our data, the majority of HSV-2 and HIV infections were acquired in the same follow-up interval (56.3%) or HIV was acquired prior to HSV-2 infection (25.0%), and only in 18.8% of co-infected participants did HSV-2 infection clearly precede HIV infection. Thus, the temporal sequence of co-infection suggests that unsafe sex places men at risk of both viral infections.

It is plausible that the association between HIV acquisition and HSV-2 may be due to confounding by high-risk sexual behaviors placing individuals at risk for both viruses. HSV-2 has much higher infectivity than HIV; the incidence of HSV-2 acquisition in this study population was 4.90/100 person-years [9], compared with an HIV incidence of 1.09 /100 person-years. The higher infectivity of HSV-2 makes it more likely to be contracted prior to HIV, so one cannot conclude that a temporal association is causal. If the association of HIV and HSV-2 is largely due to behavioral confounding, it may help to explain the lack of efficacy of HSV suppression for prevention of HIV acquisition [12,13].

It is possible that individuals may acquire HSV-2, develop ulceration, and then acquire HIV all within the same interval in this study. As 6396 individuals needed to be followed to identify 17 dual HIV and HSV-2 seroconversions, we could not obtain more frequent samples so the long testing intervals is a limitation to determining timing of infections in this study.

The finding that HIV incidence was increased with washing genitals after sexual intercourse is counterintuititive, but supports previous finding that washing the penis within 10 min of sexual intercourse increases the risk of HIV acquisition among uncircumcised men [21]. The increased HIV acquisition with penile washing may be due to the removal of acidic vaginal secretions or the addition of water with a neutral pH may assist HIV survival and infectivity.

The findings call into question the causal association between HSV-2 and HIV that has been hypothesized from prior observational studies and suggests that unsafe sex places men at risk of both viral infections. This reinforces the need for promotion of safe sex as the primary method of prevention of both HIV and HSV-2 infections, as well as other STIs.

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Table 1

Risk factors for HIV incident infection.

	No. of seroconverted/person-years	HIV incident cases/100 person-years	[0,4–5]Unadjusted analysis		[0,6–7]Adjusted analysis	
			IRR (95% CI)	P^a	IRR (95% CI)	P^a
All participants	105/9604	1.09				
[0,1–7]Age (years)						
15–19	14/2508.75	0.56	1		1	
20–24	35/2592.25	1.35	2.42 (1.30–4.50)		1.40 (0.69–2.86)	
25–29	29/1832.25	1.58	2.84 (1.50–5.37)		1.95 (0.85–4.48)	
30–49	27/2670.75	1.01	1.81 (0.95–3.46)	0.009	1.44 (0.59–3.51)	0.378
[0,1–7]Education						
No education	6/537.5	1.12	1		—	—
Primary	69/6393	1.08	0.97 (0.42–2.23)		—	—
Secondary or higher	30/2673.5	1.12	1.01 (0.42–2.42)	0.984	—	—
[0,1–7]Occupation						
Nonwage	84/6920.25	1.21	1		1	
Wage	16/1281.25	1.25	1.03 (0.60–1.76)		0.94 (0.54–1.66)	
Student	5/1402.5	0.36	0.29 (0.12–0.72)	0.027	0.65 (0.22–1.94)	0.708
[0,1–7]Marital status						
Never married	42/4317	0.97	1		1	
Currently married	50/4769.25	1.05	1.08 (0.72–1.63)		0.48 (0.26–0.89)	
Previously married	13/517.75	2.51	2.58 (1.40–4.80)	0.009	1.26 (0.60–2.70)	0.005
[0,1–7]Nonmarital relationships						
No	62/6686	0.93	1		1	
Yes	43/2918	1.47	1.59 (1.08–2.35)	0.019	1.29 (0.78–2.13)	0.520
[0,1–7]Number of sexual partners during past year						
None[b]	7/1585.5	0.44	1		—	—

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	No. of seroconverted/person-years	HIV incident cases/100 person-years	[0,4–5]Unadjusted analysis		[0,6–7]Adjusted analysis	
			IRR (95% CI)	P^a	IRR (95% CI)	P^a
1	59/5304.75	1.11	2.53 (1.16–5.54)		1	
2+	39/2713.75	1.44	3.30 (1.48–7.38)	0.013	0.77 (0.47–1.26)	0.306
[0,1–7]Condom use past year						
None	41/4050	1.01	1		1	
Inconsistent use	46/2668.25	1.72	1.71 (1.13–2.61)		1.42 (0.87–2.31)	
Consistent condom use	11/1300.25	0.85	0.84 (0.43–1.63)	0.015	0.83 (0.39–1.78)	0.205
[0,1–7]Alcohol use with sexual intercourse						
No	42/5338.5	0.79	1		1	
Yes	63/4265.5	1.48	1.89 (1.28–2.80)	0.001	1.25 (0.80–1.95)	0.324
[0,1–7]Prefer sexual intercourse with partner's vagina						
Wet during intercourse	95/7786.25	1.22	1		—	—
Dry during intercourse	3/199.25	1.51	1.21 (0.38–3.83)		—	—
No preference	0/33	0.00	0	0.950	—	—
[0,1–7]Wash genitals after sexual intercourse						
No	4/1060.25	0.38	1		1	
Yes	94/6958.25	1.35	3.64 (1.34–9.90)	0.012	3.04 (1.11–8.33)	0.031
[0,1–7]Genital ulcer disease (self-reported)						
No	85/9157.75	0.93	1		1	
Yes	20/446.25	4.48	4.86 (2.99–7.92)	<0.001	2.76 (1.65–4.62)	<0.001
[0,1–7]Urethral discharge (self-reported)						
No	94/9386.25	1.00	1		1	
Yes	11/217.75	5.05	5.04 (2.72–9.50)	<0.001	2.54 (1.02–6.31)	0.045
[0,1–7]Dysuria (self-reported)						
No	93/9251.75	1.01	1		1	
Yes	12/352.25	3.41	3.41 (1.87–6.22)	<0.001	1.17 (0.49–2.81)	0.729

	No. of seroconverted/person-years	HIV incident cases/100 person-years	[0,4–5]Unadjusted analysis		[0,6–7]Adjusted analysis	
			IRR (95% CI)	P^a	IRR (95% CI)	P^a
[0,1–7]Circumcised						
No	69/4813.75	1.43	1		1	
Yes	36/4790.25	0.75	0.52 (0.35–0.78)	0.002	0.56 (0.37–0.86)	0.008
[0,1–7]Syphilis enrollment status						
Persistent negative	101/9319.5	1.08	1		—	—
Baseline RPR positive, TPPA positive	4/284.5	1.41	1.30 (0.48–3.52)	0.610	—	—
[0,1–7]HSV-2 status						
Persistent negative	27/5408.25	0.50	1		1	
Baseline indeterminate	17/1032.25	1.65	3.30 (1.80–6.05)		2.99 (1.58–5.68)	
Prevalent positive	44/2693.25	1.63	3.27 (2.03–5.28)		2.78 (1.64–5.68)	
Seroconverter	17/470.25	3.62	7.23 (3.94–13.27)	<0.001	5.28 (2.79–9.98)	<0.001

CI, confidence interval; HSV, herpes simplex virus type 2; IRR, incidence rate ratio; RPR, rapid plasma regain; TPPA, *Treponema pallidum* particle agglutination assay.

[a] Overall *P* value for risk factor category.

[b] Only sexually active individuals included for the adjusted analysis.

Table 2

Timing of HIV and herpes simplex virus type 2 infection in men who acquired both infections during follow-up.

Sample	HIV detected	HSV-2 detected	Viral seroconversion detected first
1	Month 6	Year 1	HIV
2	Month 6	Year 2	HIV
3	Year 1	Year 2	HIV
4[a]	Year 1	Year 2	HIV
5	Month 6	Month 6	Same interval
6	Month 6	Month 6	Same interval
7[b]	Month 6	Month 6	Same interval
8	Year 1	Year 1	Same interval
9	Year 1	Year 1	Same interval
10[a]	Year 1	Year 1	Same interval
11	Year 2	Year 2	Same interval
12	Year 2	Year 2	Same interval
13[b]	Year 2	Year 2	Same interval
14	Year 2	Month 6	HSV-2
15	Year 2	Month 6	HSV-2
16	Year 2	Year 1	HSV-2

HSV-2; herpes simplex virus type 2.

[a] Month 6 sample not tested for HIV or HSV-2.

[b] Year 1 sample not tested for HIV or HSV-2.