Genital Herpes Simplex Virus Type 2 Shedding Among Adults With and Without HIV Infection in Uganda

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Background. Despite the high prevalence of herpes simplex virus type 2 (HSV-2) in sub-Saharan Africa, the natural history of infection among Africans is not well characterized. We evaluated the frequency of genital HSV shedding in HIV-seropositive and HIV-seronegative men and women in Uganda.

Methods. Ninety-three HSV-2–seropositive Ugandan adults collected anogenital swab specimens for HSV DNA quantification by polymerase chain reaction 3 times daily for 6 weeks.

Results. HSV-2 was detected from 2484 of 11 283 swab specimens collected (22%), with a median quantity of 4.3 $\log_{10}$ HSV copies/mL (range, 2.2–8.9 $\log_{10}$ HSV copies/mL). Genital lesions were reported on 749 of 3875 days (19%), and subclinical HSV shedding was detected from 1480 of 9113 swab specimens (16%) collected on days without lesions. Men had higher rates of total HSV shedding (relative risk [RR], 2.0 [95% confidence interval {CI}, 1.3–2.9]; $P < .001$); subclinical shedding (RR, 1.7 [95% CI, 1.1–2.7]; $P = .01$), and genital lesions (RR, 2.1 [95% CI, 1.2–3.4]; $P = .005$), compared with women. No differences in shedding rates or lesion frequency were observed based on HIV serostatus.

Conclusions. HSV-2 shedding frequency and quantity are high among HSV-2–seropositive adults in sub-Saharan Africa, including persons with and those without HIV infection. Shedding rates were particularly high among men, which may contribute to the high prevalence of HSV-2 and early acquisition among African women.

Keywords. genital herpes; human herpesvirus-2; HSV-2; viral shedding; Africa.

Herpes simplex virus type 2 (HSV-2) is the major cause of genital herpes and among the most frequent sexually transmitted pathogens worldwide. The incidence and prevalence of HSV-2 infection is greatest in sub-Saharan Africa (SSA), where up to 50% of incident human immunodeficiency virus type 1 (HIV) infections are attributable to HSV [1–6]. HSV-2 establishes lifelong infection and is characterized by periods of intermittent reactivation that can be associated with clinical lesions or with subclinical, asymptomatic shedding. Studies of HSV-2 shedding in cohorts in the United States detected HSV by polymerase chain reaction (PCR) in genital samples on 9%–40% of days sampled [7–11], with higher rates observed with more frequent sampling [12, 13]. Approximately 50% of the samples with detectable HSV-2 represent subclinical reactivation, which is important in the transmission of genital herpes to sex partners [14, 15] and, along with clinical reactivation, is associated with the infiltration of CD4+ T cells, which serve as targets for HIV infection in the genital tissue [16].

Despite the high prevalence of HSV-2 in SSA, the natural history of the infection among Africans has not been well characterized. Importantly, several lines of evidence suggest that HSV-2 infection in Africa may differ from that in the United States. For example, the number of HSV DNA copies in genital secretions is higher in African samples, compared with samples from the United States and South America [17]. The efficacy of acyclovir in suppressing HSV shedding may also be attenuated in African women [18]. Differences in the virulence of viral strains of African origin have been described in animal models of HSV-2 infection, as have genetic polymorphisms in a variety of host response genes [19, 20]. Finally, the high rates of HIV infection in SSA may influence the natural history of HSV-2, owing to interactions between these infections.

Because populations in SSA have the highest prevalence of HSV-2 infection and are most likely to benefit from novel interventional and prevention trials, including studies of candidate HSV-2 vaccines, there is a need to assess viral shedding in carefully characterized cohorts in this region. To address this gap, we sought to evaluate the frequency and correlates of genital HSV shedding in HIV-seropositive and HIV-seronegative men and women in Uganda.

METHODS

Study Participants

We enrolled adults ≥18 years of age who resided in Kampala, Uganda, and were referred by clinicians from the Mulago Hospital sexually transmitted diseases clinic and 4 community HIV clinics between June 2011 and January 2012. Entry criteria included HSV-2 seropositivity; HIV-seropositive persons were eligible if they were not receiving antiretroviral therapy (ART). Pregnancy, receipt of anti-HSV drugs (including acyclovir, valacyclovir, and ganciclovir), and receipt of immunosuppressive therapies (including oral steroids) were exclusion criteria. Because HSV antibody assays are not used in clinical practice in Uganda, almost all participants had a clinical history of genital herpes. However, recruitment was not focused on persons with severe disease. We sought to enroll nearly equal numbers of HIV-infected and HIV-uninfected participants. All participants provided written informed consent, and institutional review boards approved all study protocols.

Procedures

An experienced, dedicated study clinician trained participants to obtain genital swab specimens for detection of HSV at home 3 times daily for 42 consecutive days, as described previously [11, 21, 22]. Participants were provided kits with polyethylene terephthalate swabs and prelabeled vials with PCR medium for swab collection at home, and they were asked to collect swab specimens in the morning, at midday, and at bedtime. Women swabbed the cervicovaginal, vulvar, and perianal areas, and men swabbed the penile and perianal area; genital lesions, if present, were included in the area swabbed. Participants also received a diary card and were taught to record clinical signs and symptoms of genital herpes on a daily basis.

At enrollment, participants completed a standardized medical history and physical examination. At weekly clinic visits, participants returned swab samples collected at home and reviewed the diary with study staff; they also completed an interim medical history and received a brief physical examination at each visit. HIV-seropositive participants provided blood for CD4+ T-cell count and plasma HIV RNA testing at enrollment, and those not already in care were referred to local HIV clinics for management according to Ugandan Ministry of Health guidelines.

Laboratory Methods

HSV-2 antibody status was initially determined using the Focus HerpeSelect enzyme-linked immunosorbent assay, with a positive test result defined as index of at least 3.5, and subsequently confirmed by HSV Western blotting at the University of Washington [23]. Swab samples were evaluated for HSV DNA with a real-time, quantitative, fluorescent polymerase-chain reaction (PCR) assay (TaqMan) at the University of Washington virology laboratory [24–26]. Samples with ≥150 copies/mL of HSV DNA were considered positive [24].

HIV serology was determined by a commercial enzyme-linked immunoassay, CD4+ T-cell counts were measured by flow cytometry, and HIV RNA levels were measured using real-time reverse-transcription PCR (detection level, 100 HIV RNA copies/mL) at the Makerere University–Johns Hopkins University Core Laboratory in Kampala, Uganda.

Definitions

The HSV shedding rate was defined as the number of swab specimens with HSV detected divided by the total number of swab specimens. The genital lesion rate was defined as the number of days with participant-reported genital lesions consistent with HSV infection divided by the total number of days with diary information. Subclinical shedding rates were calculated as for the total HSV shedding rate, excluding swab specimens collected on days genital lesions were observed. Episodes of shedding were defined as the presence of ≥1 swab specimen with a positive PCR result and at least 2 consecutive swab samples with negative HSV PCR results before and after the episode [12, 13]. Episodes began at the midpoint between the time the first sample in the episode was collected and the time the previous sample was collected and ended at the midpoint between the time the last sample in the episode was detected and the time the subsequent sample was collected. Because of missing data, the exact duration of the episode was not always known. In these cases, the midpoint between a minimum value (which assumes that missing data were HSV negative) and a maximum value (which assumes that missing data were HSV positive) of the episode duration was used to impute episode duration. For episodes that occurred at the beginning or end of the study, we considered episode duration to be censored at the minimum duration. The quantity of shedding was measured by the number of HSV copies, as defined by the $\log_{10}$ number of HSV DNA copies/mL of PCR buffer in swab specimens with HSV detected.

Statistical Analysis

Outcomes of interest included HSV shedding rate, subclinical shedding rate, genital lesion rate, episode frequency and duration, total HSV copy number, and subclinical HSV copy number. We examined sex, age, age at sexual debut, history of genital herpes symptoms, years since first genital herpes symptoms, and HIV status as potential risk factors. Age was modeled as a continuous variable; age at sexual debut was dichotomized at the median (17 years) to avoid the influence of outliers on model estimates. The time in years since first appearance of genital herpes symptoms was categorized on the basis of previous studies [27]. Other independent variables evaluated included circumcision status for men, hormonal contraception and menses status for women, and CD4+ T-cell count and plasma HIV RNA for HIV seropositive participants. We explored best-fit groupings for CD4+ T-cell count and plasma HIV RNA level parameterization. CD4+ T-cell count was dichotomized at 350 cells/mm^3, a common cutoff for ART initiation

in Uganda; plasma HIV RNA load was dichotomized at 10 000 copies/mL on the basis of prior studies indicating that this level of viral replication is associated with a longer HSV shedding duration [13, 28]. Risk factors for total shedding, subclinical shedding, and lesion rates were evaluated using generalized estimating equations (GEE) with Poisson distribution and log link to account for within-person correlation. GEE with Gaussian distribution and identity link were used to assess risk factors for HSV $\log_{10}$ copy number outcomes. Multivariable models were constructed on the basis of the purposeful variable selection method, using entry criteria of $P < .25$ from univariate analysis and, for retention in the multivariable model, a P value of $< .10$ or the presence of confounding that resulted in >20% change in estimates [29]. Summaries of episode duration used survival analysis to account for episodes with censored durations. Episode duration was compared by HIV status, using marginal Cox models with robust standard errors to account for correlation among episodes from the same person. To evaluate the potential impact on our findings of HIV-seropositive participants with undetectable plasma HIV RNA and presumed unreported ART use [30], we conducted sensitivity analyses for each model by excluding HIV-infected participants with undetectable plasma RNA. Two-sided P values of $<.05$ were considered statistically significant. Analyses were completed using SAS, version 9.3 (SAS Institute, Cary, North Carolina).

RESULTS

Characteristics of Study Participants

Among 197 persons screened, 93 were enrolled in the study. The predominant reason for ineligibility was lack of HSV-2 antibody (n = 85). One excluded participant had atypical HSV-2 detected

by Western blot, with no HSV-2 shedding during the study; 8 were positive for HSV-1 only by Western blot, with no HSV-2 shedding during the study; 5 did not collect any swab specimens at home; 4 reported receiving ART; and 1 woman was pregnant. The median age of participants was 36 years (range, 18–67 years); 31 (33%) were men, and nearly all were Ugandan (Table 1). Fifty-four participants (58%) were infected with HIV, with a median CD4+ T-cell count of 502 cells/mm³ (interquartile range [IQR], 291–681 cells/mm³) and a median HIV RNA level of 4.8 $\log_{10}$ copies/mL (IQR, 4.0–5.2 $\log_{10}$ copies/mL) among those with detectable virus. Seventeen HIV-seropositive participants had undetectable HIV RNA levels despite self-reporting no history of ART.

Eighty-nine participants (96%) were HSV-2 and HSV-1 seropositive, while 4 (4%) were HSV-2 seropositive only. The median age at sexual debut was 17 years (range, 10–25 years). Twenty-six women (42%) reported using hormonal contraception, and 10 (32%) men were circumcised. Thirty-four participants (37%) reported a history of symptoms of oral herpes; 80 (86%) reported a history of symptoms of genital herpes, with a median time between the first clinical episode and study enrollment of 2.5 years (range, 0.1–36.6 years).

The study participants collected a median of 125 of 126 anticipated genital swab specimens (range, 19–132 swab specimens) over a median of 43 days (range, 7–45 days). A total of 11 283 genital swab specimens collected on 3869 days were included in the analysis; all 3 intended samples were collected on 3657 days (94%).

HSV Genital Shedding

HSV was detected at least once among 87 participants (94%). The overall genital HSV shedding rate from this 3-times-daily

Table 1. Demographic and Clinical Characteristics of the Study Cohort

| Characteristic | Total Cohort (n = 93) | HIV-Seronegative Group | | HIV-Seropositive Group | |
		Men (n = 11)	Women (n = 28)	Men (n = 20)	Women (n = 34)
Age, y	36 (18–67)	30 (18–48)	28 (19–58)	40 (26–67)	39 (25–56)
Male sex	31 (33)	11 (100)	. . .	20 (100)	. . .
Ugandan nationality	90 (97)	10 (91)	28 (100)	19 (95)	33 (97)
HIV seropositivity	54 (58)	. . .	. . .	20 (100)	34 (100)
CD4+ T-cell count, cells/mm³, median (IQR)[a]	502 (291–681)	. . .	. . .	292 (245–535)	582 (442–724)
HIV RNA load, $\log_{10}$ copies/mL, median (IQR)[a,b]	4.8 (4.0–5.2)	. . .	. . .	5.0 (4.5–5.2)	4.7 (3.7–5.1)
Male circumcision	10 (32)	4 (36)	. . .	6 (30)	. . .
Hormonal contraception	26 (42)	. . .	15 (54)	. . .	11 (32)
Age at first sex, y	17 (10–25)	17 (14–25)	17 (13–23)	18 (10–25)	17 (13–21)
HSV antibody status					
HSV type 2 only	4 (4)	1 (9)	0 (0)	0 (0)	3 (9)
HSV types 1 and 2	89 (96)	10 (91)	28 (100)	20 (100)	31 (91)
History of genital herpes symptoms	80 (86)	9 (82)	23 (82)	17 (85)	31 (91)
Time from first genital herpes episode to enrollment, y[c]	2.5 (0.1–36.6)	2.2 (0.3–16.8)	1.7 (0.1–34.6)	5.0 (0.1–26.6)	2.0 (0.1–36.6)

Data are median value (range) or no. (%) of subjects, unless otherwise indicated.

Abbreviations: HIV, human immunodeficiency virus type 1; HSV, herpes simplex virus; IQR, interquartile range.

[a] Among HIV-seropositive subjects.

[b] Measured only for those with detectable plasma HIV RNA.

[c] Among those reporting a history of genital herpes symptoms.

Table 2. Genital Herpes Simplex Virus (HSV) Shedding and Lesion Outcomes in the Study Cohort

HSV Outcome	Total Cohort (n = 93)	HIV-Seronegative Group		HIV-Seropositive Group	
		Men (n = 11)	Women (n = 28)	Men (n = 20)	Women (n = 34)
HSV detection frequency					
Swabs per participant, no.	125 (19–132)	125 (124–132)	126 (71–127)	124 (19–129)	125 (43–127)
Days sampled per participant, no.	43 (7–45)	43 (42–45)	43 (24–43)	43 (7–44)	43 (15–44)
Participants with ≥1 swab with HSV detected	87 (94)	10 (91)	26 (93)	20 (100)	31 (91)
Swabs with HSV detected	2484 (22)	418 (30)	463 (13)	811 (35)	792 (19)
Days with HSV detected	1169 (30)	194 (41)	236 (20)	370 (46)	369 (26)
Swabs with subclinical HSV detected	1480 (16)	263 (22)	322 (11)	343 (23)	552 (16)
Days with subclinical HSV detected	758 (24)	133 (33)	175 (17)	183 (36)	267 (22)
Lesion frequency					
Days with lesions reported	749 (19)	74 (16)	171 (14)	292 (36)	212 (15)
Shedding episodes					
Episode rate per 30 d					
All episodes	2.8 (0–10.4)	4.2 (0–6.3)	2.1 (0–7.6)	4.2 (0.7–8.7)	2.1 (0–10.4)
Subclinical episodes	1.7 (0–8.3)	2.8 (0–4.2)	1.4 (0–7.1)	2.1 (0–4.8)	1.4 (0–8.3)
Episodes with ≥1 lesion-day	0.7 (0–5.4)	0.7 (0–2.8)	0 (0–3.5)	0.7 (0–5.4)	0 (0–4.2)
Episode duration, h, median (IQR)					
All episodes	22.5 (8.5–64.8)	16.3 (8.5–64.4)	15.6 (8.3–55.0)	28.9 (12.9–123.9)	19.9 (8.4–55.0)
Subclinical episodes	14.7 (8.0–32.8)	9.2 (8.0–24.5)	10.4 (8.0–34.5)	21.3 (8.6–32.2)	15.3 (8.0–39.1)
Episodes with ≥1 lesion–day, no.	100.6 (16.4–182.3)	184.1 (16.5–272.0)	47.4 (8.5–112.7)	143.7 (25.3–175.9)	55.2 (16.1–185.3)
HSV load, log$_{10}$ copies/mL[a]					
Overall	4.3 (2.2–8.9)	4.4 (2.2–8.9)	3.9 (2.2–8.5)	4.9 (2.2–8.6)	4.2 (2.2–8.2)
Subclinical episodes	3.7 (2.2–8.7)	3.6 (2.2–8.7)	3.6 (2.2–8.5)	3.6 (2.2–8.4)	3.9 (2.2–7.8)
Lesional	5.3 (2.2–8.9)	5.6 (2.2–8.9)	4.5 (2.3–8.5)	6.3 (2.2–8.6)	4.7 (2.2–8.2)

Data are median value (range) or no. (%) of subjects, unless otherwise indicated.

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range.

[a] Among samples with HSV detected.

sampling study was 22% (2484 of 11 283 swab specimens); on 30% of days (1169 of 3869) at least one of the swab specimens was positive (Table 2). HSV was detected on 46% of days among HIV-seropositive men, on 41% of days among HIV-seronegative men, on 26% of days among HIV-seropositive women, and on 20% of days among HIV-seronegative women. To determine whether 3-times-daily sampling detected more rapidly cleared subclinical episodes than daily sampling, as shown in US studies [12, 13], we evaluated once-daily sampling by selecting only the first sample per day from each participant. Eighty-two participants (88%) had HSV detected at least once, and only 21% of days had HSV detected, illustrating that many episodes of subclinical shedding last <24 hours.

Diurnal variation in shedding frequency was not observed during the 3 periods (Supplementary Figure 1). However, the frequency of viral shedding varied widely: 6 people did not shed during the study, 31 shed on 1% to <10% of the days, 21 shed on 10% to <20%, 18 shed on 20% to <40%, 13 shed on 40% to <70%, and 4 shed on ≥70% (Figure 1).

Men had higher rates of total HSV shedding, compared with women (relative risk [RR], 2.0 [95% confidence interval {CI}, 1.3–2.9]; P < .001) in univariate analysis. Among HIV-infected participants, CD4+ T-cell count of <350 cells/μL (RR, 1.7 [95% CI, 1.0–2.7]; P = .04) and HIV plasma RNA load of >10 000 copies/mL (RR, 1.7 [95% CI, 1.0–2.8]; P = .04) were associated with increased shedding in univariate analysis. No multivariate model was identified for total HSV shedding because no model had >1 significant variable. No other factors, including HIV status, age, age at first sex, history of genital herpes symptoms, years since first genital herpes symptoms, male circumcision, menses, or use of hormonal birth control, were found to be significantly associated with total HSV shedding (Table 3).

Subclinical HSV Genital Shedding

The overall percentage of genital swab specimens with HSV collected on days without genital lesions was 16% (1480 of 9113); the percentage of days with subclinical HSV shedding was 24% (758 of 3121; Table 2). HSV subclinical shedding occurred at least once for 82 participants (92%). The percentage of days with subclinical shedding varied widely by participant (Figure 1). Overall, 65% of days with genital HSV shedding occurred on days on which no lesions were reported.

Men had higher rates of subclinical shedding, compared with women (RR, 1.7 [95% CI, 1.1–2.7]; P = .01). No other variables were associated with subclinical shedding in univariate analysis (Table 3).

Total HSV shedding, % of swabs

Subclinical HSV shedding, % of swabs

Genital lesions present, % of days
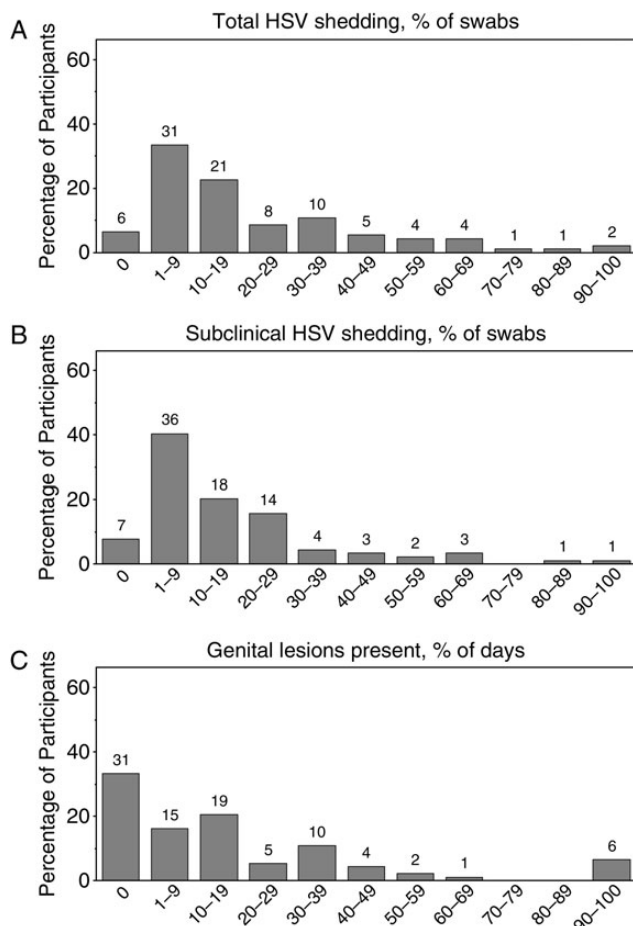
Figure 1. Distribution of participants by the percentage of swab specimens associated with total herpes simplex virus (HSV) shedding (*A*), the percentage of swab specimens associated with subclinical HSV shedding (*B*), and the percentage of days with genital lesions (*C*). The numbers above the bars refer to the absolute number of participants in each group.

Genital Lesions

The overall percentage of days with genital lesions reported was 19% (749 of 3875; Table 2). Sixty-two participants (67%) reported at least 1 day with a genital lesion; the percentage of days with genital lesions varied by participant (Figure 1). HSV was detected on 55% of days with lesions reported (411 of 748 days with lesions and HSV PCR results).

Men had higher rates of genital lesions, compared with women (RR, 2.0 [95% CI, 1.2–3.4]; $P = .009$) in univariate analysis and after adjustment for history of genital herpes symptoms (RR, 2.1 [95% CI, 1.2–3.4]; $P = .005$). History of genital herpes symptoms was also associated with increased rates of genital lesions (RR, 3.7 [95% CI, 1.4–9.5]; $P = .007$) in univariate analysis and after adjustment for sex (RR, 3.8 [95% CI, 1.5–9.8]; $P = .005$). No other factors were significantly associated with lesion frequency (Table 3).

Episode Frequency and Duration

The median frequency of shedding episodes over a 30-day period for the entire cohort was 2.8 episodes (range, 0–10.4

episodes) of HSV shedding, 1.7 episodes (range, 0–8.3 episodes) of subclinical shedding, and 0.7 episodes (range, 0–5.4 episodes) with at least 1 day of genital lesions. Of 398 total episodes, duration was known exactly for 334 (84%). The median duration of any HSV shedding episode was approximately 22.5 hours (IQR, 8.5–64.8 hours); the median episode duration was highest among HIV-seropositive men (28.9 hours [IQR, 12.9–123.9 hours]), followed by HIV-seropositive women (19.9 hours [IQR, 8.4–55.0 hours]), HIV-seronegative men (16.3 hours [IQR, 8.5–64.4 hours]), and HIV-seronegative women (15.6 hours [IQR, 8.3–55.0 hours]). The median subclinical episode length was 14.7 hours (IQR, 8.0–32.8 hours); the median subclinical episode duration was highest among HIV-seropositive men (21.3 hours [IQR, 8.6–32.2 hours]), followed by HIV-seropositive women (15.3 hours [IQR, 8.0–39.1 hours]), HIV-seronegative men (9.2 hours [IQR, 8.0–24.5 hours]), and HIV-seronegative women (10.4 hours [IQR, 8.0–34.5 hours]). The median duration of episodes with at least 1 day of genital lesions was 100.6 hours (IQR, 16.4–182.3 hours). Episode duration did not differ significantly by HIV status in unadjusted or age-adjusted analyses.

HSV DNA Copy Number

Of the 2484 genital swab specimens in which HSV-2 was detected, the median quantity was 4.3 $\log_{10}$ copies/mL (range, 2.2–8.9 $\log_{10}$ copies/mL). Presence of lesions was the only variable significantly associated with HSV copy number. Median quantity on days with lesions was 5.3 $\log_{10}$ copies/mL (range, 2.2–8.9 $\log_{10}$ copies/mL) and on days without lesions was 3.7$\log_{10}$ copies/mL (range, 2.2–8.7 $\log_{10}$ copies/mL; $\log_{10}$ change, 0.8 [95% CI, .5, 1.2]; $P < .001$). Similar results were observed in sensitivity analyses that excluded HIV-positive participants with undetectable HIV plasma RNA, although that analysis suggested that participants with CD4+ T-cell counts of <350 cells/μL had higher HSV copy numbers ($\log_{10}$ change, 0.5 [95% CI, −.02 to 1.0]; $P = .06$) when adjusting for presence of lesions.

Of the 1480 genital swab specimens in which HSV-2 was detected on days without lesions, the quantity of HSV was lower among men who were circumcised, compared with those who were uncircumcised ($\log_{10}$ change, −0.6 [95% CI, −1.0, −.1]; $P = .02$). No other factors were associated with HSV copy number in univariate analysis. Representative HSV-2 shedding and copy number patterns for HIV-positive and HIV-negative men and women are shown in Figure 2.

To evaluate the potential impact on our findings of presumed unreported ART use by HIV-seropositive participants with undetectable plasma HIV RNA [30], we conducted sensitivity analyses for each model by excluding the 17 HIV-infected participants without detectable plasma RNA. In general, the sensitivity analyses yielded results similar to those observed in the full cohort, with minor changes in point estimates and *P* values for some variables (Supplementary Table 4 and Supplementary Data).

Table 3. Risk Factors for Herpes Simplex Virus (HSV) Genital Shedding or Lesion Frequency

Risk Factor	Total HSV Shedding Unadjusted RR (95% CI)	P Value	Subclinical HSV Shedding Unadjusted RR (95% CI)	P Value	Genital Lesions Unadjusted RR (95% CI)	P Value	Genital Lesions Adjusted[a] RR (95% CI)	P Value
HIV status (seropositive vs seronegative)	1.4 (.9–2.1)	.14	1.2 (.8–1.9)	.41	1.6 (.9–2.7)	.12	. . .	
Sex (men vs women)	2.0 (1.3–2.9)	<.001	1.7 (1.1–2.7)	.01	2.0 (1.2–3.4)	.009	2.1 (1.2–3.4)	.005
Age (per y increase)	1.0 (.98–1.02)	.79	1.0 (.97–1.02)	.56	1.0 (.98–1.03)	.66	. . .	
Age at first sex, y								
10–17	1.0 (Reference)		1.0 (Reference)		1.0 (Reference)		. . .	
18–25	1.1 (.8–1.7)	.54	0.9 (.6–1.5)	.77	1.0 (.6–1.7)	.97	. . .	
History of genital herpes symptoms	1.3 (.7–2.3)	.35	1.1 (.6–2.0)	.76	3.7 (1.4–9.5)	.007	3.8 (1.5–9.8)	.005
Time since first genital herpes symptoms, y								
<1	1.0 (Reference)		1.0 (Reference)		1.0 (Reference)		. . .	
1–9	1.4 (.8–2.5)	.25	1.4 (.7–2.5)	.33	2.2 (1.0–5.0)	.06	. . .	
≥10	1.1 (.5–2.3)	.79	1.1 (.5–2.3)	.89	1.4 (.6–3.3)	.46	. . .	
Unknown	1.0 (.6–1.9)	.89	1.0 (.5–2.1)	.91	2.0 (.8–5.1)	.14	. . .	
No history of genital herpes	0.9 (.5–1.8)	.75	1.0 (.5–2.1)	.90	0.5 (.1–1.5)	.21	. . .	
Male circumcision	1.2 (.7–2.1)	.52	1.3 (.7–2.3)	.36	0.8 (.3–1.9)	.58	. . .	
Hormonal birth control[b]	1.1 (.6–2.0)	.79	1.0 (.5–1.9)	.97	0.9 (.5–1.7)	.75	. . .	
Menses	0.9 (.6–1.2)	.47	0.9 (.6–1.2)	.51	1.0 (.6–1.5)	.95	. . .	
CD4$^+$ T-cell count (<350 vs ≥350 cells/mm^3)[c]	1.7 (1.0–2.7)	.04	1.5 (.8–2.7)	.17	1.6 (.8–3.1)	.15	. . .	
HIV RNA load (>10^4 vs ≤10^4 log$_{10}$ copies/mL)[c]	1.7 (1.0–2.8)	.04	1.5 (.8–2.9)	.21	1.6 (.9–3.0)	.13	. . .	

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; RR, relative risk.

[a] Adjusted for sex and history of genital herpes symptoms.

[b] Among women only. The reference group includes those using no birth control and those using birth control other than hormonal agents.

[c] For HIV-seropositive subjects only.

DISCUSSION

To our knowledge, our study is the first to rigorously characterize viral shedding in HSV-2–seropositive Africans, revealing several important observations about the natural history of genital herpes in SSA. Genital HSV-2 was detected frequently in our cohort, with rates of total and subclinical HSV-2 shedding comparable to those in many US studies [11–13, 27, 28, 31]. Of note, men had significantly higher rates of total and subclinical shedding than women, but the differences by HIV status were smaller.

Finding higher HSV-2 shedding rates among men is in contrast to US-based studies that have shown that genital HSV shedding rates among immunocompetent women are approximately 40% higher than among immunocompetent men [24, 32]. The frequency of genital lesions among men in our study was greater than that among women, which likely contributed to higher overall shedding rates among men. The HIV-infected men in our study generally had lower CD4$^+$ T-cell counts than the HIV-infected women, which may also have contributed to the higher HSV shedding rates observed among these men. Our finding that subclinical HSV-2 copy number is lower in circumcised men, compared with uncircumcised men, is intriguing. Circumcision has been shown in multiple studies to protect men against HSV-2 acquisition [33–36], and one study has shown a transiently increased rate of HSV reactivation in the

penis following circumcision [37]. However, the data on HSV shedding by circumcision status are limited [38]. This finding may have implications for increased transmission of HSV-2 in SSA, where a greater proportion of men are uncircumcised, compared with the United States. Thus, male circumcision may also provide a benefit for male-to-female HSV-2 transmission, potentially impacting HSV-2 epidemiology if it is provided to a critical number of men in SSA.

Importantly, we did not observe differences in HSV-2 shedding rates or copy number based on HIV status, although our numbers within subsets of participants were small. US studies have observed that episodes of HSV reactivation are longer in persons with HIV infection, especially those with advanced immunosuppression [13, 28]. We also observed a trend toward longer episode length among HIV-seropositive men and women in our study. The HIV-infected participants in our study were only moderately immunosuppressed, however, and these observations may differ in persons with more marked immunosuppression.

We found that some of our HIV-infected participants had undetectable plasma HIV RNA despite a self-report of not taking ART. Based on studies that have identified ART in plasma samples of up to half of participants with undetectable HIV loads who reported no ART use [30], we assumed unreported ART use among our participants with undetectable HIV
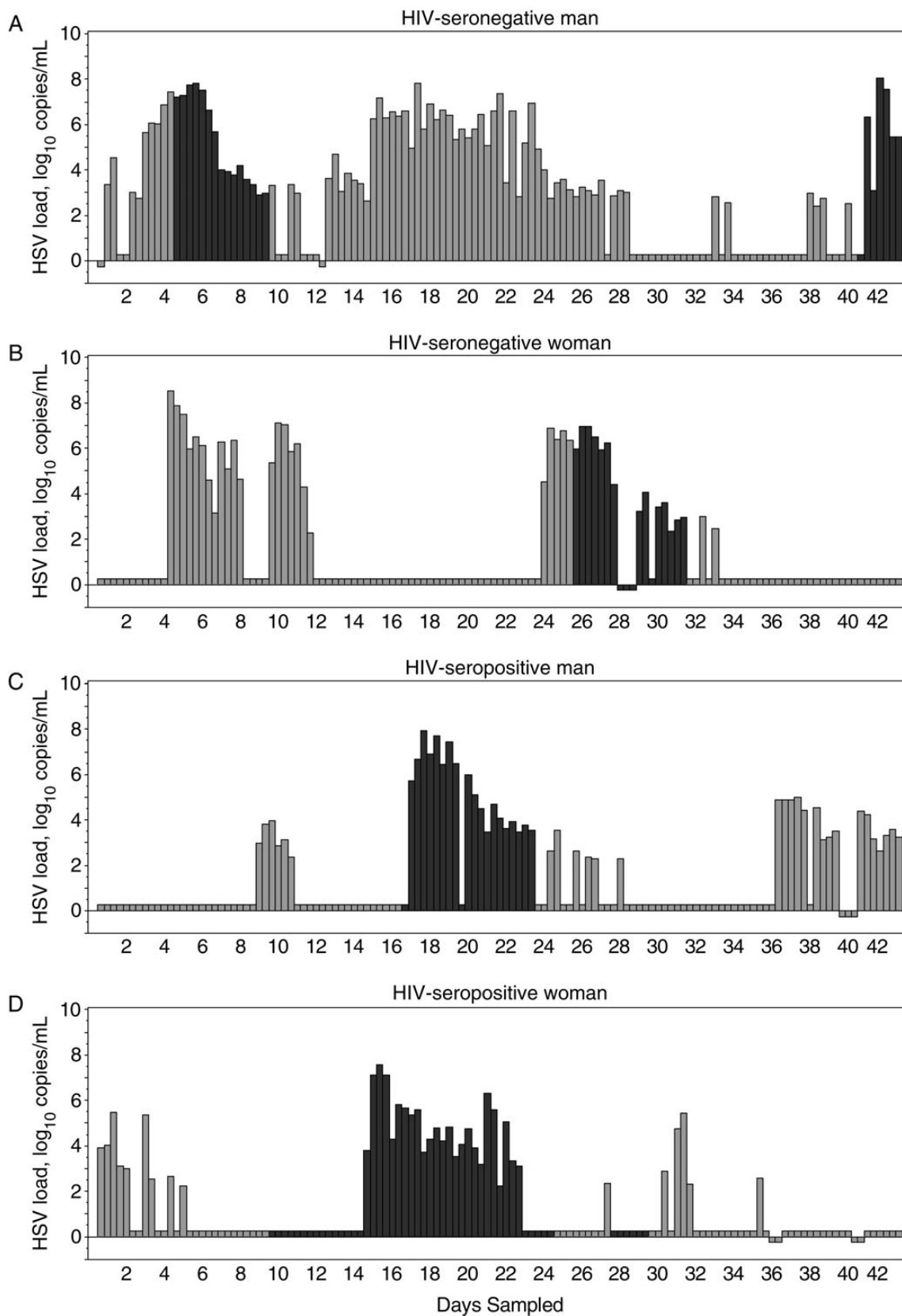
Figure 2. Representative herpes simplex virus type 2 (HSV-2) shedding patterns for 4 participants, by sex and human immunodeficiency virus (HIV) status. Black bars indicate lesions present, gray bars indicate no lesions present, and negative values indicate missing samples. *A*, Data for a 29-year-old HIV-seronegative man. *B*, Data for a 57-year-old HIV-seronegative woman. *C*, Data for a 33-year-old HIV-seropositive man with a CD4^{+} T-cell count of 273 cells/mm³ and a plasma HIV RNA load of 149 955 copies/mL. *D*, Data for a 49-year-old HIV-seropositive woman with a CD4^{+} T-cell count of 52 cells/mm3 and a plasma HIV RNA load of 290 575 copies/mL.

RNA. Sensitivity analyses excluding these participants did not appreciably alter the observed total or subclinical HSV-2 shedding rates, suggesting that ART regimens do not directly influence HSV-2 reactivation [39]. Studies of oral tenofovir similarly did not reduce HSV shedding in HIV/HSV-2 coinfected adults in Canada [40].

Our study mostly included persons with a history of symptomatic genital herpes (86% of participants), which may in part be due to enrolling patients referred from sexually transmitted disease and HIV clinics; as a result, our findings may not be generalizable to those with serologic evidence of HSV-2 infection only. Further, we only observed participants for a 6-week-long snapshot in the natural history of disease; however, participants collected genital swab specimens 3-times daily with extremely high adherence, so our findings are likely representative of the period observed. Similar to findings in the United States, the use of 3-times-daily sampling rather than once-daily sampling allowed us to capture short shedding episodes in this cohort.

In summary, our study demonstrates that HSV-2 shedding frequency, lesion frequency, and HSV-2 shedding quantity are high among HSV-2–seropositive adults in SSA, including persons with and those without HIV infection. These findings underscore the need for broad-based implementation of traditional genital herpes management strategies in SSA, including long-term antiviral medications and consistent condom use. In particular, the high shedding rates observed among men likely contribute to the high incidence of HSV-2 in Africa and underscore that men remain important targets of interventions to reduce HSV transmission. Our study also highlights the potential benefits of novel prevention interventions, including an HSV-2 vaccine, which would not only reduce the morbidity and mortality related to HSV-2 infection but could also help curtail the HIV epidemic in SSA.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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