Human Immunodeficiency Virus Acquisition Associated with Genital Ulcer Disease and Herpes Simplex Virus Type 2 Infection: A Nested Case-Control Study in Rakai, Uganda

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To assess the timing of symptomatic genital ulcer disease (GUD) relative to human immunodeficiency virus (HIV) seroconversion, we studied 248 case subjects who underwent HIV seroconversion and 496 HIV-negative control subjects, at 3 interview visits conducted at 10-month intervals: visit 1, before HIV acquisition; visit 2, after seroconversion; and visit 3, 10 months after detection of seroconversion. Odds ratios (ORs) and 95% confidence intervals (CIs), for HIV acquisition, were estimated by logistic regression. HIV load was measured by RNA–polymerase chain reaction, and herpes simplex virus type 2 (HSV-2) serologic testing used HerpeSelect EIA with Western blot confirmation. The OR of HSV-2 seropositivity associated with HIV acquisition was 1.7 (95% CI, 1.2–2.4). Prevalence of GUD was increased among case subjects, at visits 2 (OR, 3.2; 95% CI, 1.9–5.3) and 3 (OR, 2.1; 95% CI, 1.1–3.9). HIV load was increased in HSV-2–seropositive control subjects, compared with that in HSV-2–seronegative subjects, at 5 (P = .04) and 15 (P = .02) months after seroconversion. HIV acquisition is associated with HSV-2 seropositivity, and GUD is increased after seroconversion. HIV load is increased in HSV-2–positive subjects who seroconverted, suggesting a role for treatment of HSV-2 infection in HSV-2–seropositive, dually infected individuals.

Numerous observational studies have reported an association between genital ulcer disease (GUD) and an increased risk of human immunodeficiency virus (HIV) acquisition in HIV-negative individuals [1–4]. It is plausible that, in an HIV-negative individual, GUD might increase susceptibility to HIV infection by disrupting the mucosal barrier and by inflammatory changes, which increase recruitment of HIV target cells to the ulcer [1, 2]. A meta-analysis estimated the relative risk of HIV acquisition associated with GUD to be 2.7 (95% confidence interval [CI], 2.2–3.3), but there were concerns that this might, in part, reflect confounding by correlated sexual behaviors, which could increase the risk of both GUD and HIV acquisition [1]. Another potential source of confounding is that GUD could be a symptom of acute seroconversion illness. GUD is reported by 15% of individuals during the acute seroconversion illness [5] and is more common among patients with prevalent HIV [6, 7]. Thus, misspecification of the timing of reported GUD relative to the timing of HIV acquisition could result in reverse cau-
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Figure 1. Structure of data showing timing of visit and sample collection, history of genital ulcer disease (GUD), and herpes simplex virus type 2 (HSV-2) assays, relative to the timing of seroconversion.

To assess the timing of reported GUD relative to HIV seroconversion, we conducted a case-control study nested within a longitudinal cohort, in which HSV-2 serologic test results and data on GUD symptoms were available before, concurrent with, and after detection of HIV seroconversion.

PATIENTS, MATERIALS, AND METHODS

During 1994–1998, we conducted a community-randomized trial of sexually transmitted disease (STD) control for prevention of HIV in rural Rakai District, southwestern Uganda [7]. The study population comprised >10,000 initially HIV-negative individuals, 15–59 years old, from 56 rural communities. Information on behaviors, STD symptoms, and blood samples were obtained during 3 home-based interviews conducted at 10-month intervals. At each interview visit, participants were asked about genital ulceration during the previous 6 months (period prevalence) and about symptoms current at the time of the visit or within the previous 7 days (point prevalence). All participants provided informed consent at the time of enrollment and before each follow-up visit, and the study was approved by 4 institutional review boards (2 in Uganda and 2 in the United States) [7].

HIV was detected by 2 EIAs (Vironostika HIV-1; Organon Teknika and Cambridge Biotech), and discordant EIA results or new seroconversions were confirmed by Western blot (HIV-1 Western Blot; Bio-Merieux-Vitek). HSV-2 seropositivity was determined by HerpeSelect 2 ELISA IgG (for detection of human IgG-class antibodies to HSV-2; Focus), and ELISA-positive samples were confirmed by Western blot [8]. The HSV-2 assay was conducted on the blood samples obtained before HIV seroconversion. The HerpeSelect assay is considered to be positive if the index value (estimated from the ratio of optical density in the sample to the optical density of a cut-off calibrator) exceeds 1.1. However, false-positive results are known to occur, and, on the basis of results confirmed by Western blot (O. Laeyendecker, personal communication), we further categorized the index values as “probable” positives (index values, 1.1–3.5) or “definite” positives (index value, >3.5).

These data were used to design a nested case-control study. Case subjects were defined as subjects who experienced HIV seroconversion, and 2 HIV-negative control subjects were matched to each case subject, by sex, age (±2 years), and calendar time of seroconversion (i.e., incidence density sampling of HIV-negative control subjects who provided an interview and a blood sample concurrent with the visit at which the seroconversion was detected). There were 248 case subjects and 496 matched control subjects. Figure 1 shows the structure of the data. We ascertained a history of GUD from both case subjects and control subjects, at 3 interview visits: visit 1, before the period of seroconversion risk; visit 2, after seroconversion; and visit 3, 10 months after detection of seroconversion. Information was available from 246 case subjects at the first visit, from 248 case subjects at the second visit, and from 182 case subjects at the third visit. Information was available from 393 control subjects at the first visit, from 496 control subjects at the second visit, and from 349 control subjects at the third visit.

Odds ratios (ORs) and 95% CIs of HIV seroconversion associated with HSV-2 infection and/or GUD were estimated for each of the 3 periods (between enrollment and visit 1, between
visit 1 and visit 2, and between visit 2 and visit 3). Adjusted ORs were estimated by conditional logistic regression, after controlling for numbers of sex partners and condom use, reported at each interview visit. Age, sex, and timing of observation were controlled for by the matching procedure. At the first visit, any reported GUD clearly preceded HIV acquisition. The period prevalence of GUD reported at the second visit might have preceded, been concurrent with, or occurred after HIV acquisition. However, the point prevalence of GUD reported at the second visit occurred after HIV seroconversion, as did GUD reported at the third visit, which, on average, was 15 months after HIV acquisition. We hypothesized that, if GUD or HSV-2 infection were possible risk factors for HIV seroconversion, the ORs of HIV acquisition would be increased before seroconversion (i.e., at visit 1). If GUD is a cause and/or a consequence of HIV acquisition, the ORs would be increased for the period prevalence of GUD reported for the period concurrent with HIV infection (i.e., visit 2). However, if GUD was a consequence of HIV acquisition, then the ORs would be increased for the point prevalence of GUD at visit 2 and for the period prevalence of GUD reported at visit 3. Also, we hypothesized that, if HSV-2 infection was the major cause of GUD, these associations would be stronger among HSV-2–positive than among HSV-2–negative individuals. Therefore, we conducted analyses stratified by HSV-2 serostatus.

HIV load in case subjects who seroconverted was determined by reverse-transcriptase polymerase chain reaction (PCR), by use of Amplicor HIV-1 Monitor 1.5 Assay (Roche Molecular Systems), from the blood sample obtained at visit 2, when seroconversion was first detected (i.e., an average of 3 months after HIV acquisition, assuming that seroconversion occurred randomly throughout the 10-month period), and at visit 3. Because of cost considerations, virus load assays were performed on a random sample of case blood specimens, for 130 individuals at visit 2 and for 58 individuals at visit 3. To assess whether HSV-2 seropositivity affected the postseroconversion viremia, analyses were stratified by HSV-2 status.

### RESULTS

Because of the matched study design, case subjects and control subjects were comparable, with respect to age and sex. A total of 132 female (53.2%) and 116 male (46.8%) case subjects were matched with 264 female (53.2%) and 232 male (46.8%) control subjects, respectively. The median age of female subjects was 25 years (mean, 28.3 years); the median age of male case subjects was 27.0 years (mean, 30.7 years) and that of male control subjects was 27.5 years (mean, 30.6 years).

The association between timing of reported GUD and HIV acquisition is shown in table 1. The period prevalence of GUD reported at the first visit was 8.5% in the case subjects and 5.9% in the control subjects. This difference was not statistically significant (adjusted OR, 1.3; 95% CI, 0.7–2.5). However, at the second visit, the period prevalence of reported GUD was 17.3% in the case subjects and 6.3% in the control subjects (adjusted OR, 2.8; 95% CI, 1.6–5.3). At the second visit, the point prevalence of GUD after HIV acquisition was 3.2% (8/248) in the case subjects and 0.2% (1/496) in the control subjects (unadjusted OR, 16.5; 95% CI, 2.2–733.6). At the third visit, 11.6% of case subjects and 6.7% of control subjects reported GUD (adjusted OR, 2.1; 95% CI, 1.1–3.9).

We also assessed the association between HSV-2 seropositivity and HIV acquisition. The prevalence of HSV-2 was 70.2% (174/248) in case subjects and 57.9% (287/496) in control subjects (OR, 1.7; 95% CI, 1.2–2.4). The OR of HIV acquisition associated with probable HSV-2 positive values (index values, 1.1–3.5) was 1.7 (95% CI, 1.0–2.9) and that associated with definite HSV-2 positive values (index value, >3.5) was 2.1 (95% CI, 1.4–3.1).

Table 2 shows incidence of GUD reported by case subjects and control subjects at the 3 visits, stratified by HSV-2 status. Among HSV-2–seropositive case subjects, the period prevalence of GUD increased significantly between the first visit (10.5%) and the 2 subsequent visits (19.1% and 13.6%, respectively; P = .025). However, among HSV-2–seropositive control subjects, we observed no time trends in the period prevalence of

<table>
<thead>
<tr>
<th>Timing of GUD symptoms</th>
<th>No. of subjects with GUD/ no. of subjects tested (%)</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before seroconversion (visit 1)</td>
<td>21/246 (8.5)</td>
<td>23/393 (5.9)</td>
<td>1.5 (0.8–2.7)</td>
</tr>
<tr>
<td>Concurrent with or immediately after seroconversion (visit 2)</td>
<td>43/248 (17.3)</td>
<td>31/496 (6.3)</td>
<td>3.2 (1.9–5.2)</td>
</tr>
<tr>
<td>After seroconversion (visit 3)</td>
<td>21/181 (11.6)</td>
<td>24/358 (6.7)</td>
<td>2.1 (1.1–3.9)</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval; OR, odds ratio.

* Adjusted for number of sex partners and condom use, by conditional logistic regression, accounting for matching variables of age, sex, and period.
Table 2. Genital ulcer disease (GUD) reported before, during, and after the period of seroconversion risk, stratified by herpes simplex virus type 2 (HSV-2) serostatus.

<table>
<thead>
<tr>
<th>Timing of GUD symptoms, HSV-2 status</th>
<th>No. of subjects with GUD/no. of subjects tested (%)</th>
<th>OR of HIV acquisition (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before seroconversion (visit 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>18/172 (10.5)</td>
<td>1.4 (0.7–2.7)</td>
</tr>
<tr>
<td>–</td>
<td>3/74 (4.1)</td>
<td>1.3 (0.3–1.1)</td>
</tr>
<tr>
<td>Concurrent with or immediately after</td>
<td></td>
<td></td>
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<tr>
<td>seroconversion (visit 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>33/173 (19.1)</td>
<td>2.8 (1.6–5.1)</td>
</tr>
<tr>
<td>–</td>
<td>10/74 (13.5)</td>
<td>3.5 (1.4–8.9)</td>
</tr>
<tr>
<td>After seroconversion (visit 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>18/132</td>
<td>2.3 (1.1–4.7)</td>
</tr>
<tr>
<td>–</td>
<td>3/49</td>
<td>0.9 (0.2–3.3)</td>
</tr>
</tbody>
</table>

NOTE. +, Positive; –, negative; CI, confidence interval; HIV, human immunodeficiency virus; OR, odds ratio.

GUD, which remained between 6.5% and 7.8% during the 3 periods. Among HSV-2–seronegative case subjects, the period prevalence of GUD increased from 4.1%, at the first visit, to 13.5%, at the second visit, but this difference was not statistically significant (P = .67), and declined to 6.1%, at the third visit. Period prevalence of GUD reported by HSV-2–negative control subjects was low at the first 2 visits (3.1% and 4.3%, respectively) and increased to 6.9% at the third visit, but this apparent increase was based on small numbers and was not statistically different from the prevalences of GUD reported at the earlier visits (P = .19).

Table 2 also shows the associations between GUD and HIV acquisition, by HSV-2 status. At the first visit, although more case subjects than control subjects reported GUD, the differences were not statistically significant for either HSV-2–seropositive or –seronegative individuals. However, at the second visit, the period prevalence of GUD was 19.1% in HSV-2–seropositive case subjects and 7.7% in HSV-2–seropositive control subjects (OR, 2.8; 95% CI, 1.6–5.1), and period prevalence of GUD was 13.5% in HSV-2–seronegative case subjects and 4.3% in HSV-2–seropositive control subjects (OR, 3.5; 95% CI, 1.4–8.9). At the third visit, 13.6% of HSV-2–seropositive case subjects and 6.5% of HSV-2–seropositive control subjects reported GUD (OR, 2.3; 95% CI, 1.1–4.7), whereas the period prevalence of GUD was 6.1% in HSV-2–seronegative case subjects and 6.9% in HSV-2–seropositive control subjects. Thus, the higher rates of GUD among case subjects who experienced HIV seroconversion were observed during the period of seroconversion or the period 10 months after detection of HIV infection, and the excess of GUD among these subjects was mainly observed among HSV-2–seropositive case subjects.

We also determined the HIV load in case subjects who experienced HIV seroconversion (table 3). At the second visit, the log_{10} HIV load was increased among 152 HSV-2–seropositive case subjects (4.48 log_{10} copies/mL), compared with 67 HSV-2–seronegative case subjects (3.93 log_{10} copies/mL; P = .004). There were no significant differences in virus loads between case subjects reporting GUD symptoms and those not reporting GUD symptoms, irrespective of HSV-2 serostatus. However, among case subjects without symptomatic GUD, HSV-2–seropositive individuals had significantly higher virus loads (4.46 log_{10} copies/mL) than did HIV-2–seronegative individuals (3.83 log_{10} copies/mL; P = .003). At the third visit, the mean HIV loads were 4.53 copies/mL in HSV-2-seropositive subjects and 4.40 copies/mL in HSV-2-seronegative subjects (P = .6), and virus load was comparable among those reporting GUD symptoms and those not reporting GUD symptoms.

DISCUSSION

These results suggest that prevalence of symptomatic GUD is increased both during the period of HIV seroconversion risk and after HIV acquisition and that this increase is mainly observed among HSV-2–seropositive individuals (tables 1 and 2). Our data do not permit precise definition of the timing of GUD in relation to HIV acquisition, because GUD symptoms reported during the period of seroconversion risk might have occurred before HIV infection, concurrent with the acute seroconversion illness, or after seroconversion. Thus, GUD may be both a risk factor and a symptom of HIV acquisition. However, the increased point prevalence of GUD at the second visit and the increased period prevalence of GUD at the third visit suggest that symptomatic GUD is increased as a consequence of HIV infection (table 1). Moreover, the persistent increase in
GUD associated with HIV acquisition was mainly observed among HSV-2–seropositive subjects who experienced HIV seroconversion (table 2). These findings are consistent with other data indicating that herpetic GUD is a common symptom in HIV-infected individuals [5, 6, 10–13]. Thus, concurrent HSV-2 infection may explain why GUD is exacerbated by HIV infection.

HSV-2 seropositivity was high in this population (70.2% in the case subjects and 57.9% in the control subjects), and the OR of HIV acquisition associated with HSV-2 seropositivity was 1.7, which is compatible with the OR (2.1) found in a meta-analysis of prospective studies [8]. Also, in the Rakai setting, HSV-2 infection is the most common cause of GUD, detected in 44% of genital ulcers assessed by multiplex-PCR assays [7]. That symptomatic GUD was increased among HSV-2–seropositive case subjects after HIV acquisition suggests that HSV-2 infection may exacerbate prior HSV-2 infection, leading to more-symptomatic ulceration, as has been reported in other studies [10–13]. Thus, reactivated ulcerative herpes could account for the increase of GUD after HIV acquisition.

The absence of an association between HIV acquisition and GUD, at the first visit, and the ambiguous interpretation of the period prevalence at the second visit raise some questions with regard to the role of symptomatic genital ulceration as a cofactor for HIV acquisition [1–3]. Observational studies may be unable to precisely define the temporal sequence of GUD in relation to timing of HIV seroconversion, and this could lead to potential confounding by reverse causality. Such confounding might occur if a history of GUD concurrent with or after HIV acquisition was conflated with or was assumed to represent GUD symptoms before HIV infection. This potential confounding, if present, could result in overestimation of the ORs, the rate ratios, and the population-attributable fraction of incident HIV ascribed to GUD in previous analyses, by ourselves and others [12, 13] However, asymptomatic microulceration due to herpes, a common occurrence in HSV-2–seropositive individuals, also could have contributed to the risk of HIV acquisition, as suggested by the increased OR (1.7) associated with HSV-2 seropositivity. With immunosuppression associated with HIV seroconversion, such microulceration may have been exacerbated, resulting in an increased frequency of symptomatic GUD after HIV acquisition. If symptomatic ulceration is partially a consequence of HIV acquisition, the syndromic approach to GUD management in HIV-negative individuals, as a means of HIV prevention, might have less effect than previously assumed. In addition, if most GUD is caused by HSV-2 infection, treatment of bacterial STDs without treatment of HSV-2 infection, as was done in 2 published trials of STD control for prevention of HIV [7, 14, 15], may be less efficacious than previously thought.

At the second visit, subjects who experienced HIV seroconversion and who were HSV-2 seropositive had significantly higher HIV loads than did subjects who experienced HIV seroconversion who were HSV-2 seronegative, but this was not found at the third visit (table 3). Reports of GUD were not associated with a significant effect on virus load, although the power to detect an effect was limited by small numbers of individuals reporting GUD. Nevertheless, among asymptomatic subjects who underwent HIV seroconversion, HSV-2 seropositivity was associated with a significantly higher virus burden at the second visit. Thus, HSV-2 seropositivity was associated with higher viremia in the short-term, but not in the long-term. This suggests that HSV-2 infection may up-regulate HIV replication [16]. Previous studies of patients with prevalent HIV also have reported increased HIV load after active herpes ulceration [17]. Higher HIV viremia is associated with increased rates of HIV transmission per coital act, as is GUD in HIV-positive individuals [18], and GUD is increased after HIV se-
roconversion (tables 1 and 2). Thus, the enhanced HIV viremia and increased symptomatic GUD, in newly HIV-infected individuals with concurrent HSV-2 infection, may have implications for increased HIV infectivity [19]. In addition, higher postseroconversion viremia is associated with poorer prognosis and the more rapid onset of AIDS [20, 21]. This finding suggests that, for HIV and HSV-2 coinfected individuals and their partners, treatment of HSV-2 infection may provide potential benefits against active GUD and that, for HSV-2-seropositive asymptomatic individuals, this treatment may provide prophylaxis.

In conclusion, our findings suggest that symptomatic GUD is increased as a consequence of HIV acquisition, probably as a result of reactivation of preexisting HSV-2 infection. Also, there is evidence that HSV-2 up-regulates HIV load and causes GUD, suggesting a role for treatment/prophylaxis of HSV-2 infection in individuals at risk for HIV and in newly HIV-infected individuals.

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References