A Prospective Study of Genital Herpes Simplex Virus Type 2 Infection in Human Immunodeficiency Virus Type 1 (HIV-1)–Seropositive Women: Correlations with CD4 Cell Count and Plasma HIV-1 RNA Level

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A cohort of 217 human immunodeficiency virus type 1 (HIV-1)–seropositive women was observed prospectively from 1996 through 2000 to determine the frequency of genital herpes simplex virus type 2 (HSV-2) disease (symptomatic and asymptomatic) and to correlate those findings with HIV-1–related immunosuppression (absolute CD4 cell counts and plasma HIV-1 RNA levels). Participants underwent twice-yearly pelvic examinations, including cultures of cervicovaginal specimens and swab specimens from genital lesions, if lesions were present. Of the participants, 72 (33%) had genital HSV-2 infection diagnosed on the basis of either history alone (23 [32%]) or positive culture results (49 [68%]). The 72 women who had genital herpes diagnosed completed 242 total visits. Of these visits, positive HSV-2 culture results were noted at 80 (33%); at 23 (29%) of the 80 visits at which there were HSV-2–positive cultures, culture results were not associated with a clinically apparent genital lesion. Positive HSV-2 culture results occurred more frequently for samples obtained from patients with higher plasma HIV-1 RNA levels ($P = .019$) and lower CD4 cell counts ($P < .001$).

An estimated 45 million people in the United States are infected with herpes simplex virus (HSV) type 2 (HSV-2) [1]. Women are more commonly infected than men, with seroprevalences of 24.2% and 17.1%, respectively [1]. African American women have the highest reported seroprevalence (55.7%) for HSV-2 infection in the United States [1]. African American women also have the highest HIV-1 seroprevalence among women in the United States (49 cases per 100,000 African American women vs. 2.3 cases per 100,000 white women) [2].

Although coinfection with HSV-2 is common among HIV-seropositive women, little is known about the natural history of HSV-2 infection in this population. A prior study of coinfection demonstrated that asymptomatic genital HSV-2 shedding increases with lower CD4 cell counts; however, this study was limited by its cross-sectional nature [3]. To date, the effect of plasma HIV-1 RNA levels on symptomatic and asymptomatic genital HSV-2 disease has not been extensively evaluated.

Genital HSV-2 infection may have important impli-
cations for sexual transmission of HIV-1. HIV-1 has been isolated from genital herpes lesions of HIV-1–seropositive men [4] and from genital ulcers of female commercial sex workers [5]. Acquisition of HIV-1 is also influenced by prior infection with HSV-2, as reported in a recent meta-analysis that demonstrated a risk estimate of 2.1 for acquisition of HIV-1 in patients with prior HSV-2 infection [6]. In situations in which the sequence of infection could not be determined, the risk estimate was even higher (3.9) [6].

To study interactions between HIV-1–induced immunosuppression and genital HSV-2 disease, we prospectively observed a cohort of HIV-1–infected women who were assessed for cervicovaginal HSV-2 shedding twice per year, and we correlated these findings with absolute CD4 cell counts and plasma HIV-1 RNA level.

METHODS

From March 1996 through December 2000, a total of 217 HIV-1–infected women were enrolled in the 1917 Clinic (University of Alabama at Birmingham Outpatient HIV Clinic) Women’s Natural History Study, an ongoing, prospective study of women who receive primary care for HIV infection at the University of Alabama at Birmingham. Approximately 80% of the HIV-infected women who attend the 1917 Clinic are enrolled in the Women’s Natural History Study.

At the time of study entry, each woman completed a detailed questionnaire that recorded data on demographic characteristics, previous medical and obstetrical history, behavioral patterns, and history of sexual activity, HIV risk factors, injection drug use, smoking, sexually transmitted diseases, and prescription drug use. Each woman underwent standardized physical and pelvic examinations (including Papanicolaou smear) at least every 6 months. During the pelvic examination, the external genitalia and vagina were examined for evidence of lesions. Examinations were also performed on an as-needed basis to evaluate patient complaints.

Informed consent was obtained from all study participants. The project was approved by the University of Alabama at Birmingham Institutional Review Board.

Blood samples (~20 mL) were obtained by means of venipuncture at each visit for determination of absolute CD4 cell count and plasma HIV-1 RNA level (Amplisor Monitor assay; Roche). For culture for HSV, material obtained from swabs of genital secretions (or lesions, when present) was inoculated onto continuous fibroblast tissue culture cells. Monolayers were observed for appearance of viral cytopathic effects. Identification of HSV type 1 and HSV-2 in tissue culture was confirmed by direct immunofluorescence staining with type-specific monoclonal antibodies.

Descriptive statistics (including mean values, median values, SDs, and proportions) were calculated to summarize demographic characteristics, CD4 cell counts, HIV-1 RNA levels, and HSV-2 culture positivity rates. The nonparametric Wilcoxon rank sum test was used to compare continuous CD4 cell counts and HIV-1 RNA levels between visits at which HSV-2–positive culture results were obtained (hereafter referred to as “HSV-2 culture–positive visits”) versus visits at which HSV-2–negative culture results were obtained (hereafter referred to as “HSV-2 culture–negative visits”). The \( \chi^2 \) test was used to compare HSV-2 culture positivity rates across categorized CD4 cell counts and HIV-1 RNA levels.

RESULTS

For the 217 HIV-1–seropositive women enrolled in the Women’s Natural History Study, the median age was 37 years (range, 19–66 years); 69% were African American and 31% were white. HIV-1 exposure was attributed to heterosexual contact for 96% of the women and to injection drug use for 9%. At the time of enrollment, the median duration of HIV-1 seropositivity was 60.5 months, the median absolute CD4 cell count was 439 cells/mm\(^2\) (range, 4–1775 cells/mm\(^2\)), and the median plasma HIV-1 RNA level was 1851 copies/mL (range, <50–799,460 copies/mL) (table 1).

Of the 217 HIV-1–seropositive women, 72 (33%) had received a diagnosis of genital herpes. This was confirmed by a positive culture result for 49 (68%) of the 72 women. The remainder (23 [32%]) had received a clinical diagnosis without culture confirmation (figure 1A). The 72 HIV-1–seropositive women with a diagnosis of genital herpes were observed for a mean of 25 months (median, 23.5 months), and each woman...
completed a mean of 3.9 visits (median, 3.5 visits). This resulted in a total of 242 visits for the 72 HIV-1–seropositive women with a history of genital herpes (figure 1B).

Of the 242 total visits, HSV-2–positive cultures occurred for samples obtained at 80 visits (33%). Of these 80 visits, 23 (29%) occurred in the absence of a clinically apparent genital lesion, as determined by pelvic examination (figure 1B). Of these 23 episodes, 9 (11% of all HSV-2 culture–positive visits) were in completely asymptomatic women, whereas 14 were associated with various symptoms, such as perineal pain or pruritus in the absence of a clinically apparent lesion. No correlation was found between HSV-2–positive cultures and the presence of gonorrhea, chlamydial infection, trichomoniasis, bacterial vaginosis, or genital warts (data not shown). In addition, no correlation was found between HSV-2 culture positivity and the method of birth control (data not shown).

At the 80 visits at which samples that tested positive for HSV-2 were obtained, the mean (±SD) CD4 cell count was 209 ± 259 cells/mm³ (median, 107 cells/mm³), with a mean plasma HIV-1 RNA level of 171,920 ± 411,386 copies/mL (median, 21,976 copies/mL). At the 162 HSV culture–negative visits, the mean CD4 cell count was 331 ± 261 cells/mm³ (median, 308 cells/mm³), with a mean plasma HIV-1 RNA level of 100,414 ± 316,191 copies/mL (median, 4977 copies/mL). There were statistically significant differences for CD4 cell count (P = .0002) and HIV-1 RNA level (P = .004) between HSV-2 culture–positive visits and HSV-2 culture–negative visits, as determined with use of the Wilcoxon rank sum test (table 2).

When these rates of culture positivity were correlated with the degree of HIV-induced immunosuppression, a significant association was seen. Of the 80 HSV-2 culture–positive visits, one-half (n = 40) involved women with CD4 cell counts of ≥100 cells/mm³ at the time of the visit. In contrast, of the 162 HSV-2 culture–negative visits, 86 (53.1%) involved women with CD4 cell counts of ≥250 cells/mm³ at the time of the visit (P < .001) (table 3).

A similar association was seen between HSV-2 culture positivity and plasma HIV-1 RNA level. Of the 80 HSV-2 culture–positive visits, 32 (41.0%) involved women with plasma HIV-1 RNA levels of >50,000 copies/mL at the time of the visit. In contrast, of the 162 HSV-2 culture–negative visits, 81 (50.0%) involved women with HIV-1 RNA plasma levels of ≥5000 copies/mL at the time of the visit (P = .019) (table 4).

Patients reported that they were receiving suppressive anti-HSV therapy at 41 (17%) of the 242 visits. The median absolute CD4 cell counts and plasma HIV-1 RNA levels of women who were receiving suppressive anti-HSV therapy at these visits suggests that patients in this subgroup were more immuno-
Table 2. Association between results of culture for herpes simplex virus type 2 (HSV-2), CD4 cell count, and plasma HIV-1 RNA level.

<table>
<thead>
<tr>
<th>Class of visit</th>
<th>No. of visits</th>
<th>CD4 cell count, cells/mm³</th>
<th>Plasma HIV-1 RNA level, copies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2 culture positive</td>
<td>80</td>
<td>209 ± 259</td>
<td>171,920 ± 411,386</td>
</tr>
<tr>
<td>HSV-2 culture negative</td>
<td>162</td>
<td>331 ± 261</td>
<td>100,414 ± 316,191</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SD, unless otherwise indicated. HSV-2 culture-negative visits, clinic visits at which cervicovaginal samples were obtained that yielded negative culture results; HSV-2 culture-positive visits, clinic visits at which cervicovaginal samples were obtained that yielded positive culture results.

pressed than was the entire study population (median absolute CD4 cell count, 83 vs. 459 cells/mm²; median plasma HIV-1 RNA level, 1851 vs. 5243 copies/mL). The majority of the prescribed suppressive regimens consisted of either valacyclovir (54%) or acyclovir (29%). Despite the administration of suppressive antiviral treatment, positive culture results for HSV-2 were obtained at 12 (29%) of the 41 visits, and women were noted to have genital lesions at 10 (83%) of these 12 visits. Six HSV-2–positive culture results were obtained for patients who were prescribed suppressive acyclovir therapy, 5 were obtained for patients prescribed valacyclovir, and 1 was obtained for a patient receiving cidofovir for treatment of cytomegalovirus disease.

Acyclovir resistance testing was performed on 37 of the 80 HSV-2 isolates. Of these, 2 (5.4%) were shown to be acyclovir resistant (IC₅₀ >2.0 μg/mL). One of these acyclovir-resistant isolates was obtained from a patient receiving long-term immunosuppressive acyclovir therapy. The other was obtained from a patient who was receiving immunosuppressive therapy with valacyclovir after experiencing clinical failure with immunosuppressive acyclovir therapy.

**DISCUSSION**

This study provides an opportunity to evaluate the characteristics of genital HSV-2 infection among HIV-seropositive women. The demographic characteristics of this cohort of women are very typical for HIV-1–infected women in the southeastern United States, for whom the incidence of HIV-1 infection is increasing, especially among African American women [2]. The majority of our female patients acquired HIV-1 infection through heterosexual contact [2]. This contrasts with findings from studies from the northeastern or western United States, where HIV-1 transmission through injection drug use is more common [3].

This study demonstrates that culture-positive HSV-2 genital disease is strongly associated with more advanced HIV-1–induced immunosuppression, as manifested by lower absolute CD4 cell counts and higher plasma HIV-1 RNA levels. Clinic visits from patients with absolute CD4 cell counts of <100 cells/mm³ accounted for 50% of the HSV-2–positive culture results, but visits from women with this absolute CD4 cell count parameter accounted for only 36% of all study visits. These prospective data support the results of a cross-sectional study that reported a correlation between decreasing CD4 cell counts and increased likelihood of HSV-2 culture positivity [3].

In addition, we determined the effect of plasma HIV-1 RNA levels on rates of recovery of HSV-2 from the genital tract. Augenbraun et al. [7] examined the relationship between plasma HIV-1 RNA levels and genital HSV-2 culture positivity during a 4-week period but did not identify a significant association. However, that study was attempting to define the effect of HSV-2 replication (as demonstrated by positive culture and PCR results) on plasma HIV-1 RNA levels during a limited period of time. In contrast, our study evaluated the effect of plasma HIV-1 RNA levels on the rate of HSV-2 genital shedding during a 5-year period, and we demonstrated that the frequency of positive genital HSV-2 culture results is significantly higher among patients with higher plasma HIV-1 RNA levels.

Asymptomatic shedding occurred in almost one-third of the HSV-2 culture–positive visits in our study. Several studies have shown similar high rates of asymptomatic shedding in women, especially when samples were obtained daily [8–10]. Mbopi-Keou et al. [11] reported a significant correlation between HIV-1 RNA levels and HSV-2 DNA levels measured by PCR in cervicovaginal lavage samples from coinfected African women. These data have important implications with regard to virus transmission. Unprotected sexual activity places the partners of these women at risk for acquisition of HIV-1 and HSV-2, because both are being shed at higher levels than they are in women infected with only 1 of the viruses. In addition to increased asymptomatic shedding, HIV-1–seropositive women are more likely to have symptomatic

Table 3. Association between results of culture for herpes simplex virus type 2 (HSV-2) and trends in CD4 cell count.

<table>
<thead>
<tr>
<th>Class of visit</th>
<th>No. of visits</th>
<th>≤100</th>
<th>101–250</th>
<th>&gt;250</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2 culture positive</td>
<td>80</td>
<td>40 (60.0)</td>
<td>17 (21.3)</td>
<td>23 (28.8)</td>
</tr>
<tr>
<td>HSV-2 culture negative</td>
<td>162</td>
<td>46 (28.4)</td>
<td>30 (18.5)</td>
<td>86 (53.1)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. P< .001 for association between lower CD4 cell count with HSV-2 culture positivity. HSV-2 culture–negative visits, clinic visits at which cervicovaginal samples were obtained that yielded negative culture results; HSV-2 culture–positive visits, clinic visits at which cervicovaginal samples were obtained that yielded positive culture results.
HSV-2 disease, including more frequent and more severe mucocutaneous outbreaks [12, 13].

Of 37 HSV isolates tested in our study, 5.4% were found to be resistant to acyclovir in vitro. Other groups have reported rates of acyclovir resistance ranging from 11% to 17% among HIV-infected patients receiving long-term acyclovir therapy [12]. Both of the patients who had acyclovir-resistant isolates had advanced AIDS with CD4 cell counts of <50 cells/mm³, which is consistent with previous reports of disease caused by acyclovir-resistant HSV-2 [12].

There are limitations to our study. We lacked baseline HSV-2 serological data, so our study design allowed for the segregation of women on the basis of a clinical history of genital herpes. It is possible that some of these women were misdiagnosed or that we underestimated the baseline prevalence in this cohort. Serological data could have supported the clinical diagnosis and provided more information about rates of shedding among seropositive versus seronegative women [14]. Another limitation is that samples were obtained only during scheduled clinic visits or were periodically obtained from women who had developed genitourinary symptoms. Therefore, we were unable to measure the rates of shedding on days when the patients were not seen in the clinic. More frequent sampling would provide more accurate shedding data.

In summary, our study confirms that the frequency of HSV-2 genital disease increases with decreasing CD4 cell counts in HIV-1–infected women. We also demonstrated a previously undocumented correlation between the frequency of positive HSV-2 genital culture results and higher plasma HIV-1 RNA levels. Special care must be taken to monitor and appropriately treat genital HSV-2 disease in HIV-1–coinfected women, especially as their HIV-induced immunosuppression advances.

References