Overlapping Reactivations of Herpes Simplex Virus Type 2 in the Genital and Perianal Mucosa

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(See the editorial commentary by Hook, on pages 486–7.)

Background. Genital shedding of herpes simplex virus (HSV) type 2 occurs frequently. Anatomic patterns of genital HSV-2 reactivation have not been intensively studied.

Methods. Four HSV-2–seropositive women with symptomatic genital herpes attended a clinic daily during a 30-day period. Daily samples were collected from 7 separate genital sites. Swab samples were assayed for HSV DNA by quantitative polymerase chain reaction. Anatomic sites of clinical HSV-2 recurrences were recorded.

Results. HSV was detected on 44 (37%) of 120 days and from 136 (16%) of 840 swab samples. Lesions were documented on 35 (29%) of 120 days. HSV was detected at >1 anatomic site on 25 (57%) of 44 days with HSV shedding (median, 2 sites; range, 1–7), with HSV detected bilaterally on 20 (80%) of the 25 days. The presence of a lesion was significantly associated with detectable HSV from any genital site (incident rate ratio [IRR], 5.41; 95% confidence interval [CI], 1.24–23.50; P = .02) and with the number of positive sites (IRR, 1.19; 95% CI, 1.01–1.40; P = .03). The maximum HSV copy number detected was associated with the number of positive sites (IRR, 1.62; 95% CI, 1.44–1.82; P < .001).

Conclusions. HSV-2 reactivation occurs frequently at widely spaced regions throughout the genital tract. To prevent HSV-2 reactivation, suppressive HSV-2 therapy must control simultaneous viral reactivations from multiple sacral ganglia.

Herpes simplex virus (HSV) type 2 is a common infection of the genital skin and mucosa. During primary infection, HSV infects genital epithelial cells [1] and then travels via sensory nerves to the sacral root ganglion, where lifelong latency is established [2, 3]. Intermittent reactivation of HSV from the ganglia and lytic replication of virus in the epithelium is thought to result in viral shedding at the genital mucosa, with or without symptoms. Longitudinal studies in HSV-2–seropositive persons have shown that HSV reactivates in the genital tract in >90% of persons [4]. Recurrences with genital ulceration and subclinical shedding are thought to occur predominantly at the site of primary acquisition [5, 6]. The anatomic patterns of genital HSV reactivation and the resulting immune response to clear the virus may affect the risk of sexual transmission of HSV as well as the acquisition of viral copathogens, such as human immunodeficiency virus (HIV) type 1.

The detection of HSV-2 reactivation depends on the frequency of sampling and the sites sampled, as well as the population under study. Among women, the most common sites of viral shedding are the vulva, cervix, and perianal area [6]. Other studies in women in which samples from cervicovaginal, vulvar, and rectal areas were grown in viral culture showed that HSV reactivation occurs over a period of days and often involves >1 anatomic site [7]. More recent studies have used polymerase chain reaction (PCR) to detect HSV from mucosal swab samples, a technique that is 4-fold more
sensitive for HSV detection than viral culture [8]. These studies of viral shedding have used “mixed” anogenital swab samples of the entire genital region (encompassing the vulva, cervix, perineum, and perianal region in women). Although this approach efficiently identifies HSV shedding in the genital region [9], it provides little information about specific anatomic sites and patterns of reactivation.

Recent modeling studies suggest that HSV-2 reactivates at overlapping genital sites from multiple ganglia [10]. To determine the anatomic patterns of HSV reactivation and the frequency of overlapping reactivations in the presence or absence of clinically evident lesions, we conducted a study with detailed sampling of the female genitalia in a cohort of 4 women with symptomatic untreated genital HSV-2.

METHODS

Study population and data collection. Immunocompetent HSV-2–seropositive women were recruited from the University of Washington Virology Research Clinic in Seattle. Women with a history of symptomatic genital herpes were eligible to participate if they were not taking antiviral medication and were willing to be seen daily for 30 consecutive days. Participants underwent a daily detailed genital examination by an experienced clinician. If lesions were present, the lesion location was recorded on genital diagrams. Seven distinct sites were swabbed at each visit: right labia majora, right labia minora, left labia majora, left labia minora, cervix, urethra, and perianal area. Care was taken to avoid contamination from other anatomic areas during sampling. Samples were collected using Dacron swabs, which were placed into 1-mL PCR tubes containing 1× digestion buffer and stored at 2°C–8°C [8]. Participants kept a daily diary detailing genital symptoms [4]. The Human Subjects Review Committee at the University of Washington approved the study. All subjects gave written informed consent.

Laboratory methods. Serum was assayed by HSV Western blot analysis to detect antibodies to HSV-1 and HSV-2 [11]. Quantitative HSV PCR was performed on DNA extracted from genital swab samples [12].

Statistical methods. Generalized estimating equations with Poisson link and small-sample adjusted standard errors [13] were used to calculate incident rate ratios (IRRs) for the association between the number of genital sites with HSV DNA detected or the maximum log10 copy number of HSV DNA and the presence of lesions (outcome). Similar models were also used to examine whether the maximum log10 copy number of HSV DNA was associated with the number of genital sites with HSV DNA detected (outcome). Small-sample adjustment was made because only 4 women and 120 observations were used in these regressions. Two-sided P values <.05 were considered statistically significant. SAS software for Windows, version 9.1.3 (SAS Institute), was used for data analysis.

RESULTS

Characteristics of study population. Four HSV-2–seropositive, HIV-seronegative women enrolled in the study. Women ranged in age from 22 to 26 years. All participants were white. Two participants were also HSV-1 seropositive. The median age at first sexual encounter was 16 years (range, 13–19 years), and the median number of lifetime partners was 14 (range, 2–30). All participants had a history of symptomatic genital herpes, with a median of 1 year since documented primary infection (range, 254 days to 5.4 years). The median number of genital recurrences in the 6 months before study participation was 4 (range, 0–9). None of the participants took antiviral medication during the study period.

HSV shedding and lesions by anatomic site. All 4 women attended the clinic for examination and swab sample collection every day for 30 consecutive days. HSV DNA was detected in 136 (16%) of 840 swab samples and on 44 (37%) of the 120 days sampled. HSV was detected from the labial region on 27 (23%), the perianal region on 25 (21%), the cervix on 17 (14%), and the urethra on 15 (12%) of days sampled (Table 1). Of the 27 days with shedding from the labia, samples were positive from only 1 site on 6 days (22%), were positive from 2 or 3 sites on 6 days (22%), and were positive from all 4 sites on 15 days (56%).

Lesions were observed at ≥1 site on 35 (29%) of 120 days (Table 1), with 5 distinct recurrences noted by clinicians. The majority of the recurrences involved the labia (32 [91%] of 35 days with lesions). HSV was detected on 26 (74%) of 35 days when a lesion was present. There were 9 days when an ulceration was clinically present but HSV DNA was not detected; these findings reflect a prolonged healing ulcer on the right labia minora in a single participant.

Of 44 days with HSV shedding, clinically apparent lesions were present on 26 days (59%) and absent on 18 (41%). HSV shedding was confined to 1 site on 18 (41%) of the positive days, and lesions were present on 12 (67%) of those days. The perianal area was the most common location with isolated shed-

<table>
<thead>
<tr>
<th>Site</th>
<th>Days with HSV detection</th>
<th>Days with lesion present</th>
</tr>
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<tbody>
<tr>
<td>Any labial site</td>
<td>27 (23)</td>
<td>32 (27)</td>
</tr>
<tr>
<td>Cervical site</td>
<td>17 (14)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Perianal site</td>
<td>25 (21)</td>
<td>7 (6)</td>
</tr>
<tr>
<td>Periurethral site</td>
<td>15 (12)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Any site</td>
<td>44 (37)</td>
<td>35 (29)</td>
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Simultaneous HSV shedding from multiple anatomic sites. HSV was detected from >1 site on 26 (59%) of the 44 days with HSV shedding. Figure 1 shows the distribution of days on which HSV was detected from >1 site, overall and in the presence of lesions. On 20 (77%) of 26 days, HSV DNA was detected from both the right and the left vulva; lesions were present on 10 (50%) of these 20 days and absent on the other 10 (Figure 2). HSV was found at all 7 sites on 7 (27%) of the 26 days with HSV shedding. In 4 (57%) of 7 days, lesions were noted at >1 site. Detectable HSV shedding from any genital site was significantly associated with the presence of a lesion (IRR, 5.41; 95% confidence interval [CI], 1.24–23.5; \( P = .024 \)). Similarly, the lesion detection rate increased for each additional genital site positive for HSV DNA (IRR, 1.19; 95% CI, 1.01–1.40; \( P = .034 \)). A 1-log increase in maximum copy number detected from the genital region was associated with a 1.62-fold increase in the number of sites positive (95% CI, 1.44–1.82); \( P < .001 \) and with a 1.27-fold increase risk in the probability of having lesions detected (95% CI, 1.07–1.50; \( P = .006 \)). Figure 3 illustrates a representative anatomic pattern of HSV detection throughout the female genitalia throughout a 4-day period, before the development of a lesion and in the presence of a lesion. These areas of reactivation involve multiple, bilateral branches of the pudendal nerve.

Figure 4 demonstrates the shedding patterns during the 30-day observation period for 2 participants who had widespread anatomic HSV detected at high copy numbers during both asymptomatic and lesional shedding episodes. On multiple days for both women, HSV was detected from both the right and left vulva, both with and without the presence of lesions.

**DISCUSSION**

This study measured HSV-2 genital shedding patterns at defined anatomic sites in combination with a detailed daily clinical assessment for HSV-related genital signs and symptoms in women. HSV was detected from >1 anatomic site on 56% of days with any HSV shedding and was found on bilateral genital surfaces on most days on which virus was detected at >1 site. The number of sites with HSV DNA detected was associated with the maximum amount of HSV detected and the presence of a genital lesion. These results demonstrate that genital HSV reactivation may occur simultaneously from multiple sacral ganglia; the mechanism behind these observations requires further investigation. This study used a novel, very detailed sampling method in combination with a sensitive PCR assay to illustrate an important new concept in HSV-2 pathogenesis: that both clinical and subclinical HSV-2 reactivations are often multifocal and occur in a wide anatomic distribution in the genital tract. Furthermore, such reactivations are often bilateral in their anatomic distribution, even though clinical lesions typically emanate from a single anatomic focus.

In both men and women, the external genitalia are innervated by branches of the pudendal nerve, which originates from the S2, S3, and S4 ganglia of the sacral plexus [14]. It is therefore perhaps not surprising that careful sampling of the external genitalia reveals simultaneous HSV reactivations at multiple sites, given that reactivation from the ganglia that form the pudendal nerve could result in the appearance of HSV throughout the branches of the nerve distribution. What is remarkable is the high frequency of bilateral reactivations, in both the presence and the absence of lesions, perhaps due to a high frequency of simultaneous sacral ganglionic reactivation. Infection of the contralateral ganglia may occur during primary or recurrent infection. Guinea pig models have demonstrated that virus spreads from the site ipsilateral to inoculation to the contralateral dorsal root ganglia and peripheral nerves within 5 days after infection [15]. Alternatively, HSV viremia may lead to infection of contralateral sacral ganglia [16]. Results of modeling studies performed by Crespi et al [10] have suggested that episodes of HSV genital shedding measured once daily with a mixed anogenital swab sample may actually represent multiple, overlapping ganglionic reactivations, particularly in the setting of a high shedding rate. The results from the current study support this dynamic model of viral reactivation, demonstrating the detection of multiple distinct areas of simultaneous HSV reactivation throughout the genital mucosa.

The observation that HSV reactivation is widespread throughout the genital tract is intriguing, because it suggests that the
virus is rapidly cleared from some areas of the genital mucosa, whereas other areas have prolonged shedding and progress to ulceration. Mark et al [17] have shown that the HSV reactivation rate has been underestimated with once-daily sampling and that HSV reactivations last a median of 13 h. The clearance of virus from mucosal surfaces is probably dependent on a number of factors, including the amount of HSV that reaches the mucosa and local immunologic factors that facilitate viral clearance. The infiltration of HSV-specific cytotoxic T cells has been shown to be correlated with resolution of HSV-related genital ulcers [18]. Zhu et al [19] have demonstrated that HSV-specific CD8+ T cells persist at the site of a genital ulceration for ≥6 months. The persistence of activated HSV-specific T cells in areas of the genital mucosa may explain why some episodes of HSV shedding are asymptotically cleared within hours but others progress to genital lesions.

One limitation of this study was the small sample size and the unique features of our cohort. All participants had a history of symptomatic genital herpes, and 3 of the 4 participants had documented acquisition of HSV-2 within the past year, which is associated with high viral reactivation [20, 21] and high lesion rates [22]. Though we observed a relatively high proportion of days with lesions during the study period (35 [29%] of 120 days), 2 of the 4 participants with recently acquired genital herpes contributed the majority of lesion days. Despite the high lesion rate, nearly one-half of HSV shedding days were asymptomatic, a proportion similar to that in larger cohorts with symptomatic disease, reported elsewhere [7]. The frequency and distribution of widespread genital reactivation in the presence or absence of lesions in other groups (men, persons with long-standing infection, and persons with asymptomatic infection) will require further study.

Our observation that HSV was detected simultaneously in different anatomic areas may reflect cross-contamination from adjacent sites rather than distinct ganglionic reactivations. Though contamination cannot be ruled out, we believe it is unlikely, based both on the careful collection techniques performed by our clinicians and on the fact that on 18 (41%) of the days with genital shedding, HSV DNA was found from only 1 site. One approach to overcome this limitation would be to

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**Figure 2.** Pattern and amount of herpes simplex virus (HSV) DNA detected on days with >1 site positive for HSV. LMJ, left labia majora; LMN, left labia minora; PA, perianal; RMJ, right labia majora; RMN, right labia minora. White, no HSV-2 detected; blue, 10^2 log_{10} copies/mL HSV DNA detected; yellow, 10^3 log_{10} copies/mL HSV DNA detected; orange, 10^4 log_{10} copies/mL HSV DNA detected; green, 10^5 log_{10} copies/mL HSV DNA detected; red, 10^6 log_{10} copies/mL HSV DNA detected; black, 10^7 log_{10} copies/mL HSV DNA detected.
use localized tissue biopsy specimens to demonstrate the simultaneous detection of HSV antigen or specific immune response in widely separated anatomic areas. We have initiated a study to explore this issue further.

These data should inform how patients are counseled about the risks of HSV transmission. Patients should be aware that one is unlikely to be able to predict not only when but also where HSV shedding will occur and that shedding may not be restricted to areas where lesions are or have been present. The relationship between shedding frequency and extent and the risk of transmission to sexual partners has not been quantified.

In conclusion, we demonstrate that HSV-2 reactivation occurs frequently and at widely spaced anatomic regions throughout the genital tract in women with a history of symptomatic...
genital herpes, suggesting that latent HSV-2 ganglionic infection is present in bilateral sacral ganglia and that control of viral replication at the level of the sacral ganglia is incomplete. Furthermore, these data suggest that the genital skin and mucosa play an essential immunologic role in clearance of HSV-2. Whether widespread subclinical reactivation occurs in HSV-2–seropositive persons without a clinical history of HSV-2, or in persons with long-standing HSV-2 infection, requires further study. However, these patterns of widespread reactivation may help explain the role of HSV-2 in increasing the risk of HIV-1 acquisition, particularly if each reactivation episode elicits a persistent immune response to clear the virus. To prevent HSV-2 transmission and the possible acquisition of viral copathogens such as HIV-1, HSV-2 therapy will need to suppress simultaneous viral reactivations from bilateral sacral ganglia more effectively.

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References