

## REACTIVATION OF GENITAL HERPES SIMPLEX VIRUS TYPE 2 INFECTION IN ASYMPTOMATIC SEROPOSITIVE PERSONS

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### ABSTRACT

**Background** Most persons who have serologic evidence of infection with herpes simplex virus (HSV) type 2 (HSV-2) are asymptomatic. Historically, it has been assumed that these persons have less frequent viral reactivation than those with symptomatic infection.

**Methods** We conducted a prospective study to investigate genital shedding of HSV among 53 subjects who had antibodies to HSV-2 but who reported having no history of genital herpes, and we compared their patterns of viral shedding with those in a similar cohort of 90 subjects with symptomatic HSV-2 infection. Genital secretions of the subjects in both groups were sampled daily and cultured for HSV for a median of 94 days.

**Results** HSV was isolated from the genital mucosa in 38 of the 53 HSV-2-seropositive subjects (72 percent) who reported no history of genital herpes, and HSV DNA was detected by the polymerase-chain-reaction assay in cultures prepared from genital mucosal swabs in 6 additional subjects. The rate of subclinical shedding of HSV in the subjects with no reported history of genital herpes was similar to that in the subjects with such a history (3.0 percent vs. 2.7 percent). Of the 53 subjects who had no reported history of genital herpes, 33 (62 percent) subsequently reported having typical herpetic lesions; the duration of their recurrences in these subjects was shorter (median, three days vs. five days;  $P < 0.001$ ) and the frequency lower (median, 3.0 per year vs. 8.2 per year;  $P < 0.001$ ) than in the 90 subjects with previously diagnosed symptomatic infection. Only 1 of these 53 subjects had no clinical or virologic evidence of HSV infection.

**Conclusions** Seropositivity for HSV-2 is associated with viral shedding in the genital tract, even in subjects with no reported history of genital herpes. (N Engl J Med 2000;342:844-50.)

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SEROLOGIC surveys indicate that the prevalence of infection with herpes simplex virus (HSV) type 2 (HSV-2) among adults approaches 25 percent in the United States and ranges from 4 to 18 percent in western Europe.<sup>1-5</sup> In most studies, only 10 to 25 percent of subjects with HSV-2 infection report a history of genital lesions.<sup>1,2</sup> Historically, it has been assumed that persons with asymptomatic HSV infections have less frequent and less severe reactivations than those with symptomatic disease. However, two lines of evidence suggest this may not be true. First, many HSV-2-seropositive subjects who initially report having no history of genital

lesions do, after an educational session with a clinician, subsequently report having such lesions.<sup>6,7</sup> Such subjects most likely have unrecognized symptomatic infection. Second, most HSV-2 infections are acquired from a person with no history of genital herpes infection.<sup>8,9</sup> These data suggest that viral shedding in seropositive subjects may be frequent, regardless of the presence or absence of a reported history of genital herpes.

To investigate viral shedding in HSV-2-seropositive persons who were asymptomatic, we identified HSV-2-seropositive women and men who reported no history of genital lesions. After they attended an educational session on the symptoms and signs of genital herpes, the subjects were followed with daily cultures of the genital area to evaluate the frequency and the sites of viral shedding in the genital tract. The clinical and virologic characteristics of the infection in these subjects were compared with those in subjects with symptomatic genital HSV-2 infections who followed a similar regimen.<sup>10</sup>

### METHODS

#### Study Subjects

We recruited 37 subjects with no reported history of genital herpes infection from among persons who were identified as being seropositive for HSV-2 in a random screening for HSV antibodies among patients attending a primary care clinic,<sup>11,12</sup> but who reported having no history of genital herpes, and 16 subjects who were recruited as potential candidates for a study of an experimental HSV-2 vaccine but who were unexpectedly found to be positive for HSV-2 antibodies.<sup>13</sup> The study was conducted at the University of Washington Family Medicine Clinic and Virology Research Clinic in Seattle and was approved by the human-subjects review committee of the University of Washington. All subjects gave written informed consent.

All subjects attended an individual standardized educational session on genital herpes that included reviewing photographs of herpetic lesions. Photographs of both typical lesions (e.g., blisters and ulcers) and atypical lesions (e.g., fissures) were shown, and the common symptoms (e.g., itching and tingling) were discussed. The women were taught to obtain swab specimens of the cervicovaginal, vulvar, and perianal areas for viral cultures, as described previously.<sup>10,14</sup> The men were taught to obtain swabs of the penile skin, perianal area, and the urethral meatus or a first-morning urine sample for viral culture.<sup>15-17</sup> The subjects were asked to collect samples daily for three months. The subjects kept a diary of symptoms and

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signs of genital disease and were asked to come to the clinic if they noted lesions consistent with the presence of genital herpes as well as for regular monthly visits.

We also studied 90 subjects (44 men and 46 women) with symptomatic genital herpes who were HSV-2-seropositive and who were attending the Virology Research Clinic in Seattle or Westover Heights Clinic in Portland, Oregon. These subjects attended the same type of educational session and followed the same daily sampling protocol as the 53 HSV-2-seropositive persons with no reported history of genital herpes. The demographic and clinical characteristics of these 90 subjects were similar to those of the large cohort of subjects with symptomatic genital herpes whom we have studied in the past two decades.<sup>18,19</sup>

**Laboratory Methods**

Serum samples were tested for HSV type 1 (HSV-1) and HSV-2 antibodies by Western blot analysis.<sup>20</sup> Cultures for viral studies were placed in transport medium and delivered to the laboratory three times per week. Viral isolation was performed as previously described; all isolates were confirmed and typed with monoclonal antibodies.<sup>21,22</sup> HSV DNA was measured by a quantitative real-time fluorescence-based polymerase-chain-reaction (PCR) assay.<sup>23</sup>

**Study End Points**

Herpes lesions were defined as blisters, ulcers, or crusts in the genital or perianal area or on the buttocks. A recurrence was defined as the presence of a lesion at any of these sites on one or more consecutive days. Episodes of viral shedding were defined as the finding of a positive culture on one or more consecutive days. The rate of viral shedding was calculated as the number of days with a positive culture divided by the total number of days on which cultures were obtained. The analysis of subclinical shedding was based only on findings obtained on days on which lesions were absent.

**Statistical Analysis**

The chi-square and Wilcoxon rank-sum tests were used to compare the characteristics of the 53 subjects who reported having no

history of genital herpes with those of the 90 subjects with symptoms. Log-linear models were fitted to examine predictors of viral shedding. Scale factors were estimated to account for greater variability than predicted by the Poisson model.<sup>24</sup> P values for comparisons of the percentage of days with shedding between the subjects with no reported history of genital herpes and the subjects with such a history were obtained from multivariate log-linear models and, hence, are adjusted for age and sex. All statistical tests are two-sided.

**RESULTS**

**Characteristics of the Subjects**

The median age of the 53 HSV-2-seropositive subjects (42 women and 11 men) who reported no history of genital herpes was 38 years; 89 percent were white. Twenty of these subjects had antibodies to HSV-2 only, and 33 had antibodies to both HSV-1 and HSV-2. These subjects supplied swabs for viral culture on a median of 98 days. The analysis included 5887 days on which cultures were obtained and more than 17,700 viral cultures from these 53 subjects.

**Recognition of Genital Herpes**

After education and counseling, 26 of the 42 women (62 percent) and 7 of the 11 men (64 percent) who had reported having no history of genital herpes reported having typical ulcers, blisters, or crusts in the genital area during follow-up; 19 of these 33 subjects reported more than one recurrence of genital herpes. Examples of two symptom diaries are shown in Figure 1. Thirteen subjects never reported lesions but did report localized genital symptoms. In 7 of these 13 subjects, HSV-2 was identified in the genital tract

	Day																															
HSV-2-Seropositive Woman	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
No symptoms	X	X	X	X	X					X															X					X	X	X
Itching, burning, tingling								X				X	X	X	X	X	X	X				X	X			X	X		X			
Localized redness or sore spots						G	G	G	G			B	B	B	B	B	B	B	P	P	P											
Sores, blisters, ulcers, crusts												B	B	B	B	B	B	B	B	B												
Abrasion, skin splits, scratches, fissures																				P												
Thigh or buttock pain or sensitivity										X																						
Swollen groin or lymph nodes																																
Results of HSV culture												2	2		2	2	2															
HSV-2-Seropositive Man																																
No symptoms	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	
Itching, burning, tingling																																
Localized redness or sore spots																			G	G												
Sores, blisters, ulcers, crusts																																
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Swollen groin or lymph nodes																																
Results of HSV culture																	2	2														

**Figure 1.** Sample Symptom Diaries Kept by an HSV-2-Seropositive Woman and an HSV-2-Seropositive Man with No Reported History of Genital Herpes.

The woman had antibodies to HSV-1 and HSV-2. During the study, a typical HSV lesion developed on her buttock. During this episode, HSV-2 (2) was isolated initially from the buttock lesion and then from the cervix, vulva, and perianal area. The lesions were located in the genital area (G), buttocks (B), and perianal area (P). The man also had antibodies to HSV-1 and HSV-2. Even after an educational session, the man did not report having typical recurrences. He had an episode of asymptomatic shedding of HSV-2 from the penile skin on days 16 and 17 and mild, nonspecific genital symptoms on days 18 and 19.

**TABLE 1.** CORRELATION BETWEEN THE RECOGNITION OF GENITAL HERPES LESIONS AND VIRAL SHEDDING IN 53 HSV-2-SEROPOSITIVE SUBJECTS WITH NO REPORTED HISTORY OF GENITAL HERPES.

VARIABLE	HERPES SIMPLEX VIRUS RECOVERED*		
	YES	NO	TOTAL
	no. of subjects		
Genital ulcers, blisters, or crusts	28	5	33
Localized genital itching or soreness	10	3	13
No clinical evidence of genital herpes	6	1	7
Total	44	9	53

\*Herpes simplex virus was isolated from culture or identified with use of a polymerase-chain-reaction assay for viral DNA.

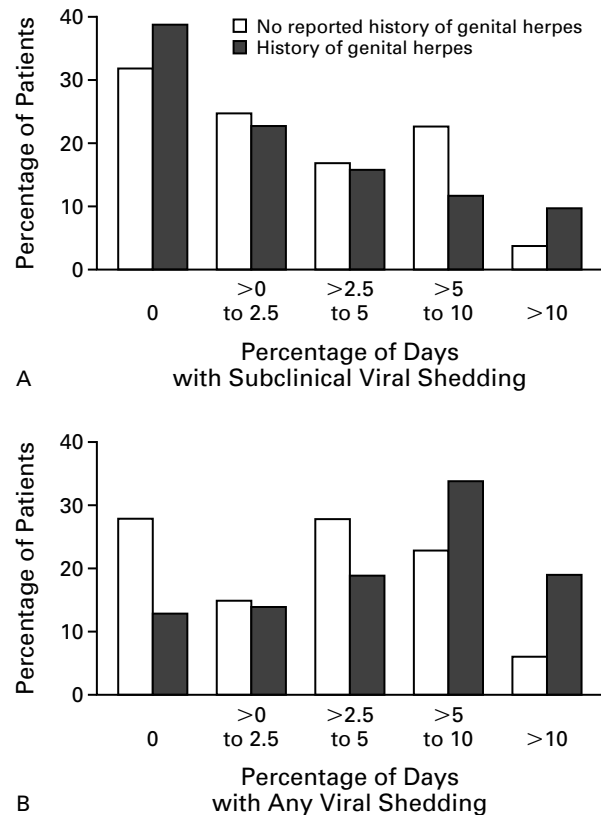
when symptoms were present. Thus, 46 of the 53 HSV-2-seropositive subjects (87 percent) who reported no history of genital herpes reported having either genital lesions or localized genital symptoms during follow-up (Table 1).

**HSV Shedding in the Genital Area**

HSV was isolated by viral culture of swabs from the genital mucosa at least once in 38 of the 53 HSV-2-seropositive subjects (72 percent) who reported no history of genital herpes. HSV-2 was isolated from 37 subjects, and HSV-1 was isolated from 1 woman who was seropositive for both HSV-1 and HSV-2. Of these 38 subjects, 36 had HSV isolated from genital mucosal swabs obtained on days on which lesions were absent (asymptomatic shedding), whereas 18 subjects had HSV isolated on days on which lesions were reported. Among the seven subjects who reported having no symptoms or signs of genital herpes during the study, HSV-2 was isolated from four and HSV-1 from one. Among the 13 subjects who reported only localized genital symptoms but no lesions, HSV-2 was isolated from 9.

Overall, among the 53 HSV-2-seropositive subjects who reported having no history of genital herpes at the time of enrollment, HSV was isolated on 3.8 percent of days on which cultures were obtained (range, 0 to 17 percent). HSV was isolated on 168 of the 5591 days (3.0 percent) on which cultures were obtained in the absence of lesions. Of the 36 subjects who had subclinical viral shedding, HSV was isolated on up to 5 percent of days on which lesions were absent in the case of 22 subjects and on more than 5 percent of days in the case of the remaining 14 subjects (Fig. 2A).

The sensitivity of the PCR assay for the detection



**Figure 2.** Frequency of Subclinical Viral Shedding (Panel A) and Any Viral Shedding (Panel B) in HSV-2-Seropositive Subjects with No Reported History of Genital Herpes and in Those with Such a History, as Defined by Isolation of the Virus in Tissue Culture.

of HSV is greater than the sensitivity of culture.<sup>23,25</sup> We investigated whether mucosal HSV infection could be demonstrated by the PCR assay in 9 of the 15 subjects from whom HSV was not isolated by standard viral-culture techniques. In six of these nine subjects (four women and two men), HSV DNA was detected by the PCR assay in swabs of genital secretions. The median number of days on which HSV DNA was detected in these six subjects was 3.5 (range, 2 to 11). The geometric mean number of copies of HSV DNA was 8700 per milliliter of PCR specimen (range, 500 per milliliter to 5 million per milliliter). Thus, overall, HSV was detected in genital secretions by either viral culture or PCR assay in 44 of 53 HSV-2-seropositive subjects (83 percent) who reported having no history of genital herpes (Table 1). Only 1 of 53 subjects had no clinical or virologic evidence of HSV infection.

**Clinical and Virologic Characteristics in Relation to History and Symptoms**

Among the 90 subjects (46 women and 44 men) with a history of genital herpes, the frequency of sero-

**TABLE 2.** CHARACTERISTICS OF HSV-2-SEROPOSITIVE SUBJECTS WITH NO REPORTED HISTORY OF GENITAL HERPES AND OF THOSE WITH SUCH A HISTORY.

CHARACTERISTIC	NO REPORTED HISTORY OF GENITAL HERPES		HISTORY OF GENITAL HERPES	
	WOMEN (N=42)	MEN (N=11)	WOMEN (N=46)	MEN (N=44)
No. of days on which cultures were obtained				
Median	101	85	82	86
Range	30-219	30-304	34-172	30-375
Viral shedding — no. of subjects (%)	30 (71)	8 (73)	42 (91)	36 (82)
Subclinical viral shedding — no. of subjects (%)	29 (69)	7 (64)	32 (70)	23 (52)
Recurrence — no. of subjects (%)	26 (62)	7 (64)	36 (78)	34 (77)
Viral shedding during a recurrence — no. of subjects (%)	14 (33)	4 (36)	35 (76)	29 (66)
Percentage of days with viral isolation	3.8	3.9	8.3	4.7
Range	0-15	0-17	0-36	0-23
Percentage of days with viral isolation in the absence of lesions	3.0	3.0	3.6	2.0
Range	0-10	0-13	0-35	0-19
Percentage of days with lesions	3.9	4.5	18.0	11.5
Range	0-25	0-25	0-72	0-53
Rate of recurrence per year				
Median	2.8	4.3	8.5	6.6
Range	0-12	0-21	0-30	0-26
Duration of recurrences — days				
Median	3	4	6	5
Range	1-17	1-11	1-53	1-22
Percentage of days with positive cultures without lesions	78	80	35	37

positivity for both HSV-1 and HSV-2 was lower than that among the subjects with no reported history of genital herpes (40 percent vs. 62 percent,  $P=0.02$ ). The symptomatic subjects were also younger (median age, 33 vs. 38 years;  $P=0.003$ ). They were predominantly white (92 percent). They supplied swabs for viral culture on a median of 84 days.

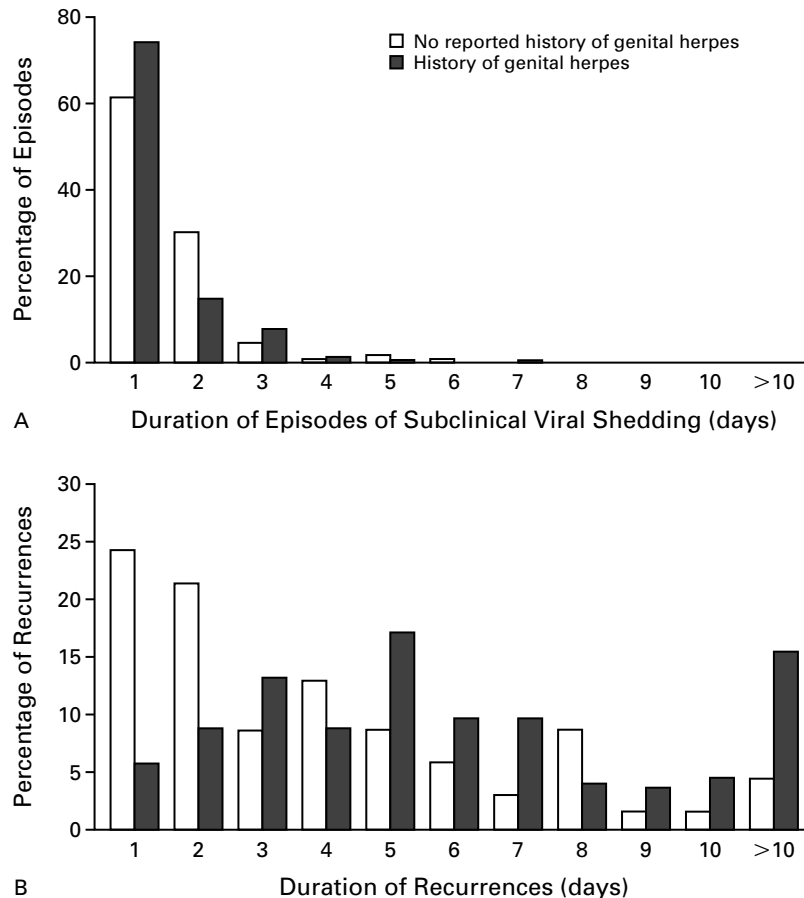
The rates of reactivation of HSV infection among the two groups of subjects are shown in Table 2 and Figure 2. The total rate of viral shedding was significantly higher among subjects with a history of genital herpes than among HSV-2-seropositive subjects with no reported history of genital herpes (6.4 percent vs. 3.8 percent of days,  $P=0.001$ ). Of all the days on which HSV was isolated in each group of subjects, 36 percent were days on which no lesions were reported among subjects with a history of genital herpes, as compared with 79 percent among the subjects with no reported history. However, the rates of subclinical viral shedding (2.7 percent vs. 3.0 percent) (Fig. 2A), the duration of episodes of subclinical shedding (Fig. 3A), and the site-specific rates of subclinical shedding were similar in the group with a history of genital herpes and the group with no reported history.

Among the women, the site-specific rates of subclinical viral shedding were 1.6 percent for the cervicovaginal area for those with a history of genital herpes

and 0.9 percent for those with no reported history of genital herpes; 1.4 percent and 1.2 percent, respectively, for the vulvar area; and 1.5 percent and 1.6 percent, respectively, for the perianal area. Among the men, the site-specific rates were 1.1 percent for the penile skin for those with a history of genital herpes and 1.9 percent for those with no reported history and 0.9 percent and 0.7 percent, respectively, for the perianal area.

The recurrence rate was significantly higher among subjects who had a history of genital herpes than among those with no reported history of genital herpes (median, 8.2 per year vs. 3.0 per year;  $P<0.001$ ) and the recurrences lasted longer (median, five days vs. three days;  $P<0.001$ ) (Fig. 3B).

In an analysis of risk factors for viral shedding, adjusted for age and sex, the HSV-2-seropositive subjects with a history of genital herpes had a significantly higher risk of viral shedding than those with no reported history (risk ratio, 1.84; 95 percent confidence interval, 1.27 to 2.66) (Table 3). The risk of viral shedding was slightly higher among women than men and tended to decrease with age. In this analysis, age may be serving as a surrogate for the time since the initial infection, which was unknown for subjects with no reported history of genital herpes, because the rates of viral shedding decrease over time among



**Figure 3.** Duration of Episodes of Subclinical Shedding (Panel A) and Recurrences of Genital Herpes (Panel B) in Subjects with No Reported History of Genital Herpes and in Those with Such a History.

symptomatic subjects.<sup>10,26</sup> In contrast to the risk of any viral shedding, the adjusted risk of subclinical shedding in the subjects with a history of genital herpes did not differ significantly from those with no reported history of genital herpes (risk ratio, 0.95; 95 percent confidence interval, 0.59 to 1.53).

### DISCUSSION

There has been controversy regarding the biologic and clinical meaning of asymptomatic HSV-2 infection. At present, the medical and public health communities largely ignore persons who have asymptomatic HSV-2 infection because little information is available regarding the benefit of identifying such persons. We systematically evaluated the rates of reactivation of infection among asymptomatic HSV-2-seropositive subjects. We found that 83 percent of subjects who were HSV-2-seropositive but who reported having no history of genital lesions had genital shedding of HSV during follow-up. Although much of the shedding was subclinical, once identified as

seropositive by serologic testing and educated about their HSV-2 infection, 62 percent of such subjects reported having typical herpetic lesions. The pattern, sites, and frequency of subclinical reactivation of infection in these subjects were similar to those in subjects with symptomatic infections.

In this study, as in prior studies,<sup>6,7</sup> knowledge of their seropositivity for HSV-2 combined with education regarding the clinical manifestations of genital herpes resulted in the recognition of typical lesions among most subjects with “silent” HSV-2 infections. The subjects’ lack of recognition of recurrent genital herpes may be explained by the lower frequency and shorter duration of lesions among subjects with silent infections, as compared with those with clinically evident genital herpes.<sup>10</sup> Although perception clearly has an important role in the diagnosis of this disease, the reported frequency or duration of episodes noted by the subjects was unlikely to have been underestimated, given the intensive follow-up and frequent visits required by our protocol. Subjects with symptomatic

**TABLE 3.** PREDICTORS OF VIRAL SHEDDING, AS MEASURED BY VIRAL CULTURE, IN SUBJECTS WITH HSV-2 INFECTION.\*

CHARACTERISTIC	UNIVARIATE RISK RATIO (95% CI)	ADJUSTED RISK RATIO (95% CI)†
<b>Any viral shedding</b>		
History of genital herpes (vs. no reported history)	1.70 (1.17–2.46)	1.84 (1.27–2.66)
Women (vs. men)	1.30 (0.90–1.87)	1.43 (0.99–2.06)
Age (per year of age)	0.98 (0.96–0.99)	0.99 (0.97–1.00)
Seropositivity for HSV-1 and HSV-2 (vs. HSV-2 alone)‡	0.90 (0.63–1.28)	—
<b>Subclinical shedding</b>		
History of genital herpes (vs. no reported history)	0.90 (0.57–1.42)	0.95 (0.59–1.53)
Women (vs. men)	1.48 (0.91–2.39)	1.33 (0.79–2.25)
Age (per year of age)	0.98 (0.96–1.00)	0.98 (0.96–1.01)
Seropositivity for HSV-1 and HSV-2 (vs. HSV-2 alone)‡	0.99 (0.63–1.56)	—

\*CI denotes confidence interval.

†The risk ratios have been adjusted for age and sex.

‡The difference between seropositivity for both HSV-1 and HSV-2 and seropositivity for HSV-2 alone was not significant in any model, so it was omitted from the multivariate models used to obtain adjusted risk ratios.

infection typical of those enrolled in treatment trials are at the more severe end of the clinical spectrum of HSV-2 infection.<sup>27-29</sup>

Whether host or viral factors are responsible for the difference in clinical and virologic manifestations of infection between subjects with frequent reactivation of HSV and those with infrequent reactivation is not known. However, the rates of subclinical viral shedding were similar among the subjects with previously unrecognized genital herpes and those with recognized infection. HSV is often transmitted during episodes of subclinical shedding<sup>8,30</sup>; with regard to infectivity, HSV-2–seropositive persons who initially reported having no lesions differed little from HSV-2–seropositive persons who recognized the lesions. Our findings concerning the potential for the transmission of HSV to sexual partners are therefore not comforting to either patients or providers.

Asymptomatic shedding of HSV has been investigated predominantly among women. Because women may shed virus “internally” (i.e., from the cervix and vagina), the idea that some reactivations of infection may go unnoticed appears plausible. In contrast, the anatomy of the genital tract in men and the fact that the genital epithelium is predominantly skin and not mucosa have made less plausible the concept of asymptomatic shedding from men’s genital skin. This issue was illustrated in a study of physicians’ attitudes toward asymptomatic shedding of HSV,<sup>31</sup> in which both male and female physicians tended to dismiss the possibility of asymptomatic shedding in men as anatomically implausible. We found that this reasoning is

erroneous; the rates of subclinical shedding among men approximated those among women.

Although our study included more than 17,700 viral cultures from HSV-2–seropositive persons with no history of genital herpes, all cultures were obtained from a total of 53 such subjects. Since studies such as ours are difficult and time consuming for the subjects, only subjects who were particularly concerned about HSV-2 infection and who were able to comply with the procedures participated. However, since most of the asymptomatic subjects in this study were initially recruited in a general medical clinic and did not present with genitourinary symptoms, it seems unlikely that the cohort was biased toward those with more severe subclinical HSV infections.

Prevention of the spread of HSV to neonates and sexual partners will require the identification and control of infection in persons with subclinical HSV infection. Although accurate, type-specific serologic tests have been available in research laboratories for more than a decade, commercially available assays have been developed and marketed only fairly recently.<sup>32-36</sup> Thus, it is just becoming possible to identify the large reservoir of persons with infrequent, short episodes of HSV-2 reactivation. Our data suggest that such persons often may not require antiviral chemotherapy for clinical symptoms and signs of infection because their episodes are short and infrequent. However, they do require education and counseling regarding their risk of transmitting the infection to others.

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