

VIROLOGIC CHARACTERISTICS OF SUBCLINICAL AND SYMPTOMATIC GENITAL HERPES INFECTIONS

ANNA WALD, M.D., M.P.H., JUDITH ZEH, PH.D., STACY SELKE, M.A., RHODA L. ASHLEY, PH.D.,
AND LAWRENCE COREY, M.D.

Abstract *Background.* The frequency, pattern, and anatomical sites of subclinical shedding of herpes simplex virus (HSV) in the genital tract, along with factors that predict such shedding, have not been well characterized.

Methods. We studied prospectively the clinical and virologic course of genital herpes in 110 women. The women kept symptom diaries and provided daily samples from the vulva, cervix, and rectum for viral culture.

Results. During a median follow-up of 105 days, subclinical shedding of virus was identified in 36 of 65 women (55 percent) with HSV type 2 (HSV-2), in 16 of 31 women (52 percent) with HSV type 1 (HSV-1) and HSV-2, and in 4 of 14 women (29 percent) with only HSV-1. Among women with genital HSV-2 infection, subclinical shedding occurred on a mean of 2 percent of the days. The mean duration of viral shedding during subclinical episodes was 1.5 days, as compared with 1.8 days during symptomatic episodes. HSV was isolated from several sites in the genital tract and rectum in 17 percent of subclinical episodes and 22 percent of symptomatic epi-

sodes. Half the episodes of subclinical shedding of HSV occurred within seven days of a symptomatic recurrence. The risk of subclinical shedding increased with the frequency of symptomatic recurrences. Subclinical shedding was more frequent among women with more than 12 recurrences per year than among those with no symptomatic recurrences (odds ratio, 3.3; 95 percent confidence interval, 1.4 to 7.9); it was also more frequent among women who had recently acquired genital herpes (odds ratio for women with HSV acquired in the past year as compared with those who had had the infection for a year or more, 1.85; 95 percent confidence interval, 1.1 to 3.1).

Conclusions. Among women with a history of genital herpes infection, subclinical shedding of HSV is common and accounts for nearly one third of the total days of reactivation of HSV infection in the genital tract. Women with frequent symptomatic recurrences also have frequent subclinical shedding and may be at high risk for transmitting HSV. (*N Engl J Med* 1995;333:770-5.)

SUBCLINICAL shedding of herpes simplex virus (HSV) occurs in persons with a history of genital herpes and is instrumental in transmitting HSV infection to sexual partners and neonates.¹⁻⁷ The rate of subclinical shedding among persons with recurrent genital herpes and the factors associated with such shedding are largely undefined. The most important factor influencing the detection of subclinical shedding is the frequency of sampling, since episodes of reactivation have been considered infrequent and brief.⁸ We conducted a prospective study of a large cohort of women with a history of symptomatic genital herpes infection who provided daily samples for viral cultures from genital sites and the rectum.

METHODS

Subjects, Setting, and Procedures

Between 1987 and 1992, we recruited otherwise healthy women with a history of genital herpes infection specifically for a study of the frequency of subclinical and symptomatic viral shedding. The entry criteria included a clinical history of genital herpes and HSV infection confirmed by Western blot analysis.⁹ All the women were counseled about the clinical signs and symptoms of genital herpes and were taught techniques of genital self-examination to detect lesions¹⁰; the women used Dacron swabs to collect vulvar, cervicovaginal, and rectal samples for viral cultures.⁸ The swabs were immersed in viral transport medium, refrigerated immediately after collection, and delivered to the laboratory at least three times each week. The women recorded the dates of onset and the duration of genital lesions in diaries and were seen in the clinic every four to six weeks and at the

time of recurrences. At these visits, the diaries were collected, and transport medium and new diary cards were dispensed. The women were asked to obtain samples from the genital tract and rectum for at least 60 consecutive days. None of the women took acyclovir for the suppression of genital herpes during the study. The study was approved by the Human Subjects Review Committee at the University of Washington.

Laboratory Methods

Antibodies to HSV were detected by Western blot assay.⁹ Samples were inoculated in triplicate into wells of human diploid fibroblast cultures in 48-well microtiter plates. The wells were examined every day for three days, then every other day for two weeks. Cultures with evidence of cytopathic effects were confirmed to contain HSV with type-specific immunofluorescence.¹¹

Statistical Analysis

Subclinical shedding was defined by the isolation of HSV from the cervix, vulva, or rectum in the absence of genital or perianal lesions noted by the subjects or the clinicians. To calculate the rate of subclinical shedding, we used the number of days without genital lesions on which secretions for culture were obtained as the denominator.

An episode of subclinical or symptomatic shedding was defined as one or more consecutive days on which HSV was isolated. Thus, if a single day with a negative or missing culture interrupted a sequence of positive cultures, the sequence was treated as two episodes rather than one. Since this definition tended to shorten the duration of an episode, we also used an alternate definition that treated a single negative culture or missing culture as positive if there were positive cultures on the previous and succeeding days; we assumed that if there were missing cultures for up to seven days between two positive cultures, those cultures were also positive. This definition tended to lengthen the duration of an episode.

A recurrence was defined as one or more consecutive days on which genital lesions were present. A recurrence was called culture-positive if HSV was isolated on any day during the recurrence, and culture-negative otherwise. Data on 50 women who provided samples for cultures both within seven days of a recurrence and more than seven days before or after a recurrence and who had subclinical shedding at least once were used to describe the temporal association of subclinical and symptomatic episodes. Potential predictors of subclinical viral shedding were assessed by means of logistic-regression analysis,

From the Departments of Medicine (A.W., L.C.), Statistics (J.Z.), Laboratory Medicine (S.S., R.L.A., L.C.), and Microbiology (L.C.), University of Washington, Seattle. Address reprint requests to Dr. Corey at the Virology Division, University of Washington, Rm. 9301, 1200 12th Ave. South, Seattle, WA 98144.

Supported by a grant (AI-30731) from the National Institutes of Health. Dr. Wald is the recipient of an American Social Health Association Postdoctoral Research Fellowship.

with the cultures for each woman grouped together. The variability among women was greater than expected with the classic logistic-regression model, so a scale factor was used to account for this variation in assessing the significance of predictors.¹²

RESULTS

Study Population

Altogether, 110 women with a clinical history of genital herpes participated in the study. Their median age was 30.5 years (range, 16 to 53); 94 percent of the women were white. Fourteen (13 percent) had genital HSV type 1 (HSV-1) infection; 96 (87 percent) had genital HSV type 2 (HSV-2) infection, of whom 65 (59 percent of the study population) had antibodies only to HSV-2 and 31 (28 percent) had antibodies to both HSV-1 and HSV-2. Twelve women had been followed in the clinic since the initial episode of genital herpes, and the remaining women had a clinical history of genital herpes. The median length of time from the acquisition of genital herpes infection to enrollment in the study was 462 days (range, 1 to 9803). The demographic characteristics of the women and the observed rates of recurrences of genital herpes were similar to those described by Benedetti et al.¹³

We followed the women for a median of 105 days (range, 5 to 799). Specimens were obtained for culture on a median of 82 days (range, 5 to 714) and a total of 12,335 days (Table 1). Overall, women provided specimens for culture on 85 percent of the study days. Forty-six women provided specimens for periods of up to 60 days without having lesions, 39 women for 61 to 120 days, 14 women for 121 to 180 days, and 11 women for more than 180 days. The median number of sites sampled daily was three; overall, 31,197 specimens were cultured and analyzed for this report.

To assess the reliability of viral cultures of specimens obtained by subjects at home as compared with that of specimens obtained by the clinical staff at the research clinic, we compared the rates of isolation of HSV on 1360 days on which specimens were obtained by both techniques. The rate of concordance for the results was 97.4 percent. HSV was isolated from 4.3 percent of the cultures of samples obtained by patients, as compared with 3.5 percent of those of samples obtained by clinic staff (P = 0.05 by McNemar's test).

Frequency of Subclinical Shedding of HSV

Of the 110 women, 56 (51 percent) had at least one day of subclinical reactivation of HSV. Subclinical shedding of HSV was identified in 36 of the 65 women with HSV-2 infection (55 percent), 16 of the 31 with both HSV-1 and HSV-2 infection (52 percent), and 4 of the 14 with HSV-1 infection alone (29 percent). Overall, subclinical shedding was documented on 2.0 percent of the days in women with genital HSV-2 and 0.7 percent of the days in those with genital HSV-1 (Table 1). Among the women, shedding occurred on 0 to 35 percent of days sampled (Fig. 1).

The rate of detection of subclinical reactivation reflected the number of days on which samples were ob-

Table 1. Frequency of Reactivation of HSV-1 and HSV-2 on Days with and Days without Genital Lesions, According to Serotype and Site of Specimen for Culture, among 110 Women.

SITE AND SEROTYPE	TOTAL DAYS	FREQUENCY OF ISOLATION OF HSV	
		WITH GENITAL LESIONS	WITHOUT GENITAL LESIONS
		<i>days with shedding/total days (%)</i>	
Any site			
HSV-1 or HSV-2	12,335	396/1506 (26.3)	186/10,829 (1.7)
HSV-1	2,517	30/80 (37.5)	17/2437 (0.7)
HSV-2	9,818	366/1426 (25.7)	169/8392 (2.0)
Vulva			
HSV-1 or HSV-2	12,116	260/1455 (17.9)	75/10,661 (0.7)
HSV-1	2,503	26/80 (32.5)	11/2423 (0.5)
HSV-2	9,613	234/1375 (17.0)	64/8238 (0.8)
Cervix			
HSV-1 or HSV-2	11,326	66/1352 (4.9)	71/9974 (0.7)
HSV-1	2,055	7/51 (13.7)	5/2004 (0.2)
HSV-2	9,271	59/1301 (4.5)	66/7970 (0.8)
Rectum			
HSV-1 or HSV-2	7,755	132/1079 (12.2)	73/6676 (1.1)
HSV-1	2,237	9/79 (11.4)	9/2158 (0.4)
HSV-2	5,518	123/1000 (12.3)	64/4518 (1.4)

tained. Sixty-three percent of the women who provided specimens for up to 60 days without having lesions never had subclinical shedding, as compared with 39 percent of the women who provided samples for more than 60 days. Table 2 shows the rates of subclinical shedding for 64 women who provided samples for at least 60 days. The 60-day duration of sampling was selected to eliminate both falsely low and falsely high rates of shedding due to short periods of sampling. Thirty-five of 54 HSV-2-seropositive women (65 percent) had subclinical shedding during a median sampling period of 106 days (range, 61 to 425), whereas 19 women (35 percent) did not have viral shedding despite a median sampling period of 97 days (range, 63 to 307). Eleven percent had shedding on more than 5 percent of the days on which samples were obtained. The rate of subclinical shedding was similar in the HSV-2-seroposi-

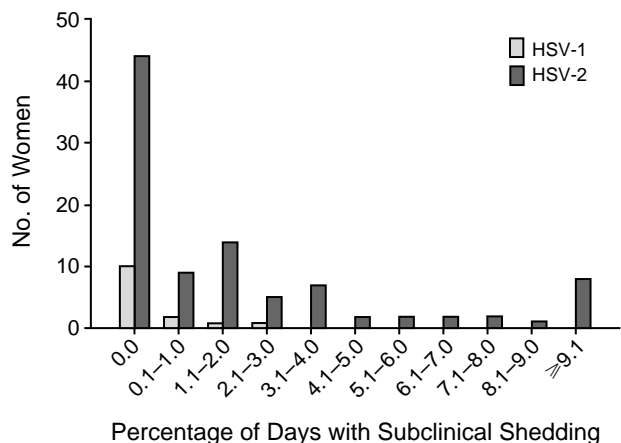


Figure 1. Frequency of Subclinical Shedding of HSV in the Genital Tract or Rectum among 110 Women. Fourteen women had only HSV-1, and 96 had HSV-2 (31 of whom also had HSV-1).

tive women and those who were seropositive for both HSV-1 and HSV-2.

Virologic Characteristics of Subclinical Shedding

Subclinical shedding occurred on 32 percent of the total days when viral shedding was detected. Of the 128 episodes of subclinical shedding, 96 (75 percent) lasted for one day, 18 (14 percent) for two days, 7 (5.5 percent) for three days, and 7 (5.5 percent) for four or more days. The durations of clinically recognized and unrecognized episodes of viral shedding are shown in Table 3. Analysis of the data with use of an alternative definition, according to which days without culture results were treated as positive, revealed a similar pattern; 70 percent of the episodes of unrecognized shedding of HSV lasted one day, 15 percent two days, 4 percent three days, and 11 percent four days or longer.

Subclinical shedding occurred at all the anatomical sites sampled. The rates of isolation of HSV from cultures of samples obtained on days when genital lesions were absent were 0.7 percent for the vulva, 0.7 percent for the cervix, and 1.1 percent for the rectum (Table 1). Nineteen of 56 women who had subclinical shedding (34 percent) shed the virus from more than one site on the same day. The HSV subtype was the same on all days on which HSV was isolated from more than one site. Shedding from more than one site occurred on 32 of 186 culture-positive days (17 percent). The most common sites of dual shedding were the vulva and cervix, which accounted for 19 days. Other episodes of shedding from multiple sites involved the vulva and rectum (nine days), the cervix and rectum (two days), or all three sites (two days).

Virologic Characteristics of Symptomatic Shedding

Episodes of symptomatic shedding of HSV were documented in 72 of the 110 women (65 percent): 5 of the 14 women with only HSV-1 infection (36 percent), 43 of the 65 women with HSV-2 infection alone (66 percent), and 24 of the 31 women with both HSV-1 and HSV-2 infection (77 percent). The median rate of recurrence per year was 0 among women seropositive only for HSV-1 and 7.5 among women who had genital HSV-2 infection. Since 37 percent of the episodes of genital lesions were culture-negative, the average number of episodes of symptomatic shedding was 6.5 per year (Table 3). HSV was isolated from two of the sampled sites on 18 percent of the culture-positive days when symptoms were present and from three sites on 4 percent of such days. The most common sites for such

Table 2. Rates of Subclinical Shedding among 64 Women Who Provided Samples for Culture on More Than 60 Days, According to Serotype.

SEROTYPE	NO. OF WOMEN	RATE OF SHEDDING (%)		
		MEAN	MEDIAN	RANGE
HSV-1	10	0.5	0	0-2.8
HSV-2	33	1.6	1.1	0-11.4
HSV-1 and HSV-2	21	2.2	0.6	0-10.3

Table 3. Virologic Characteristics of Subclinical and Clinical Episodes of HSV Shedding among 110 Women.

CHARACTERISTIC	WITHOUT GENITAL LESIONS	WITH GENITAL LESIONS
No. of episodes	128	219
Mean no. of episodes/yr*	3.8	6.5
Mean no. of days of shedding/yr*	5.5	11.7
Mean duration of episodes (days)	1.5	1.8
No. (%) of episodes		
Lasting 1 day	96 (75.0)	125 (57.1)
Lasting 2 days	18 (14.1)	51 (23.3)
Lasting 3 days	7 (5.5)	24 (11.0)
Lasting \geq 4 days	7 (5.5)	19 (8.7)
Days of shedding at \geq 1 site (%)	17	22

*Calculated as 365 times the total number of episodes or days, divided by the total number of days with positive cultures; the totals include all 110 women.

multiple shedding were the vulva and rectum (39 days) and the vulva and cervix (21 days).

Association between Subclinical and Symptomatic Shedding of HSV

To evaluate the temporal relation between subclinical and symptomatic episodes, we calculated the rate of subclinical shedding within seven days of a symptomatic recurrence. The rate of subclinical shedding was 4.9 percent within seven days of a recurrence (6.5 percent in the seven days before and 3.5 percent in the seven days after a recurrence), as compared with 1.8 percent on the days more than seven days from a recurrence. Thirty percent of the episodes of subclinical shedding occurred in the seven days preceding a symptomatic recurrence and 20 percent in the seven days after such a recurrence. Figure 2 illustrates the anatomical distribution and duration of subclinical and symptomatic shedding and the relation between these types of shedding in four representative subjects.

Predictors of Subclinical Viral Shedding

In a univariate analysis, women infected with HSV-2 or with both HSV-1 and HSV-2 had viral shedding more frequently than women infected only with HSV-1 (odds ratio, 2.8; 95 percent confidence interval, 1.1 to 7.5; and odds ratio, 3.1; 95 percent confidence interval, 1.1 to 8.5, respectively) (Table 4). Women who had acquired genital herpes within 12 months of enrollment were also more likely to have subclinical shedding than women who had been infected for a longer time (odds ratio, 2.1; 95 percent confidence interval, 1.2 to 3.7). The likelihood of subclinical shedding also increased with the annual number of recurrences. Women with more than 12 recurrences per year had subclinical shedding more often than women with no recurrences in the past year (odds ratio, 5.1; 95 percent confidence interval, 2.2 to 11.8). The rate of subclinical shedding among women with 1 to 12 recurrences per year did not differ significantly from that of women without recurrences. Age did not influence the rate of subclinical shedding.

In a multivariate analysis, frequent recurrences and

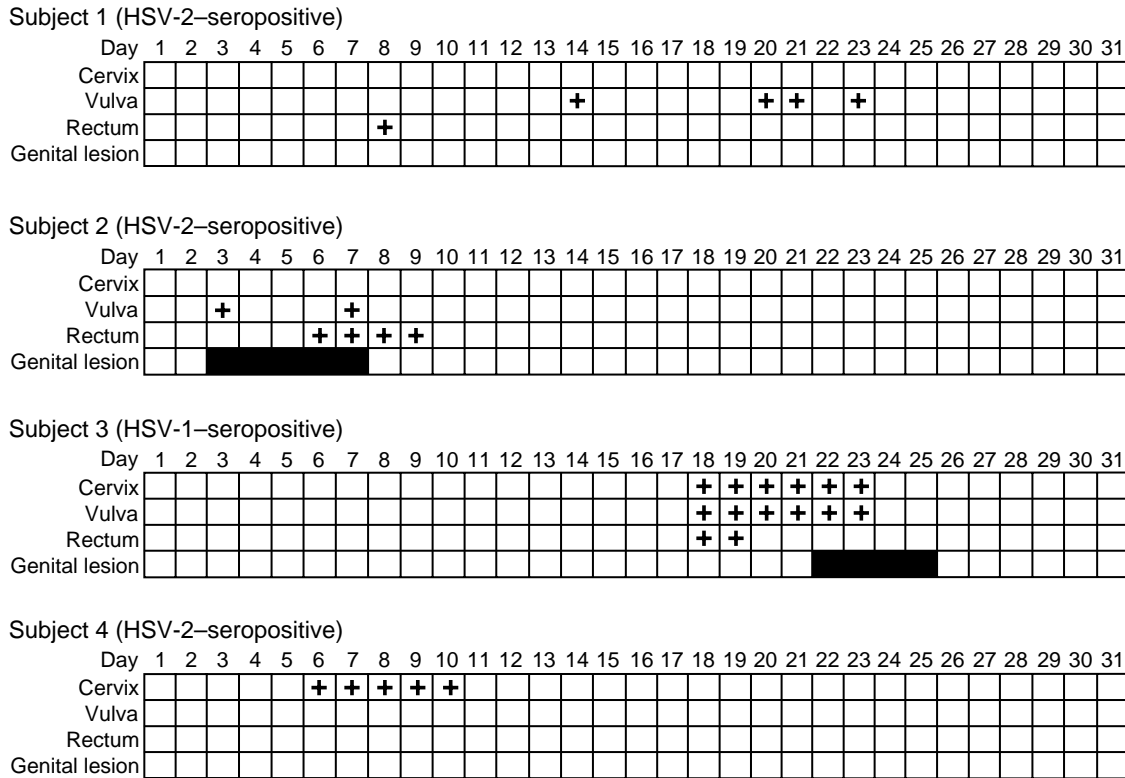


Figure 2. Pattern of Shedding of HSV during 31 Days in Four Women.

Plus signs indicate positive HSV cultures, empty boxes negative cultures, and solid boxes genital lesions. Subject 1 had intermittent, subclinical vulvar and rectal shedding. Subject 2 had subclinical shedding from the rectum closely following the healing of a perineal lesion. Subject 3 had subclinical shedding from multiple sites shortly before a symptomatic recurrence on the vulva. Subject 4 had a prolonged episode of subclinical cervical shedding.

the recent acquisition of genital herpes were independent predictors of subclinical reactivation. Women with more than 12 recurrences per year had significantly more episodes of subclinical shedding than women with no recurrences (odds ratio, 3.3; 95 percent confidence interval, 1.4 to 7.9), suggesting that these women had high rates of both symptomatic and subclinical reactivation of HSV. Women who had acquired genital herpes within a year before enrollment also had more frequent shedding (odds ratio, 1.85; 95 percent confidence interval, 1.1 to 3.1) than the women who had had genital herpes for a year or more.

DISCUSSION

To elucidate the frequency and pattern of subclinical shedding of HSV, we conducted a prospective study of a large cohort of women with a history of genital herpes. Subclinical shedding of HSV accounted for nearly one third of the total days of reactivation of genital herpes. Episodes of subclinical shedding occurred in clusters of days, similar to episodes of symptomatic reactivation; many episodes involved more than one anatomical site, and they were most likely to occur shortly before or after a symptomatic reactivation. Although subclinical shedding of HSV-2 occurred on 2 percent of the days sampled overall, 11 percent of the women with HSV-2 infection had subclinical shedding on more than 5 percent of the days, suggesting that a

subgroup of women with HSV-2 may have a high likelihood of transmitting HSV.

The proportion of women who had subclinical viral shedding in our study (51 percent) and the rate of subclinical shedding were higher than previously reported.

Table 4. Characteristics Associated with High Rates of Subclinical Shedding among 110 Women with Genital Herpes.

CHARACTERISTIC	NO. OF WOMEN	CRUDE ODDS RATIO	ADJUSTED ODDS RATIO (95% CI)*
Age (per year)	110	0.97	0.99 (0.96-1.02)
Serotype			
HSV-1†	14	1.0	1.0 (—)
HSV-2	65	2.8	1.7 (0.7-4.55)
HSV-1 and HSV-2	31	3.1	1.75 (0.6-5.0)
Time since acquisition of HSV‡			
≥1 year†	62	1.0	1.0 (—)
<1 year	40	2.1	1.85 (1.1-3.1)
No. of recurrences/yr			
0†	29	1.0	1.0 (—)
1-6	27	0.95	0.9 (0.4-2.3)
7-12	27	1.3	1.15 (0.4-3.0)
>12	27	5.1	3.3 (1.4-7.9)
No. of culture-positive recurrences/yr§			
0†	38	1.0	1.0 (—)
1-6	32	1.5	1.3 (0.6-3.4)
7-12	24	1.9	1.4 (0.6-3.4)
>12	16	10.8	6.7 (3.0-15.4)

*CI denotes confidence interval.
 †The reference category.
 ‡The time of acquisition of genital herpes was unknown for eight women.
 §Adjusted values are from a separate multivariate analysis in which the rate of culture-positive recurrences was used in place of the recurrence rate.

ed^{8,14-19} and reflected the relatively long study period. Our results indicate that there is great variability in the rates of subclinical shedding among women with genital herpes. Significant correlates of subclinical shedding included a short time since the acquisition of genital herpes and a high frequency of symptomatic recurrences. The HSV serotype, which was a significant predictor of the rate of subclinical shedding in the univariate analysis,¹⁴ was no longer significant after adjustment for the recurrence rate.

Daily sampling of the genital tract and rectum for a prolonged period allowed us to observe patterns of subclinical reactivation of HSV. Our preliminary studies showed that samples obtained by the patients were at least as reliable for the detection of HSV as samples obtained by clinicians. Thus, we feel that the patterns of subclinical shedding we observed were not affected by the sampling technique. The daily sampling and reporting methods allowed us to characterize all symptomatic recurrences, not just those that were severe enough to prompt a visit to a medical provider or participation in a clinical trial of treatment for genital herpes.

The pattern of subclinical shedding that we observed also helps to explain the wide range of shedding frequencies in previous studies of asymptomatic shedding.^{8,14-20} The duration of sampling, biologic variation in the expression of the infection, and the varying length of episodes all appear to influence the observed rates of subclinical shedding. The rates of shedding were likely to vary most among women with few days of sampling, with most having no episodes of shedding and a few with extremely high rates of shedding, depending on whether a prolonged shedding episode happened to be included in the days sampled.

Our cohort of women was selected for their willingness to provide daily samples for culture and to adhere to the protocol. Demographic characteristics, recurrence rates, and duration of HSV before enrollment in this group were similar to those of women with symptomatic genital herpes whom we have studied over the past decade.^{13,14,21} However, this type of study should be carried out in other HSV-2-seropositive populations. The pattern of subclinical shedding among men also requires characterization.

Our definition of subclinical viral shedding included shedding on all days on which the subject did not note a lesion. From the standpoint of patient education, perhaps the term "unrecognized genital herpes" is the best description of what we have termed "subclinical herpes." It is possible that some episodes of shedding from the cervical or vulvar area were associated with small lesions that would have been apparent on colposcopic examination or that were associated with non-specific symptoms such as itching, without a noticeable lesion or ulcer.^{10,15,22,23} The high rate of subclinical shedding from the perianal area can also be explained in part by the difficulty of visualizing lesions. Although it is possible that more frequent visits to a clinician would have resulted in the identification of a greater number

of genital lesions, daily visits to the clinic were not feasible. Furthermore, it is the recognition of genital lesions by the patient that may be relevant in preventing transmission to sexual partners.

Clinical and Epidemiologic Implications

Recommendations for preventing the transmission of genital herpes to sexual partners have focused on abstinence from sexual intercourse during symptomatic recurrences. However, studies of the transmission of HSV indicate that most new infections are acquired from partners with unrecognized or subclinical disease.¹⁻⁷ The seroprevalence of HSV-2 in the adult population has increased from 16 percent to 22 percent in the past decade²⁴ (and Johnson RE: personal communication). Emphasis on the importance and high frequency of unrecognized reactivation of HSV may be necessary to contain the current epidemic of genital herpes.

In summary, our data indicate that most women with symptomatic HSV-2 infection also had subclinical viral shedding and that women with frequent symptomatic recurrences also had frequent subclinical reactivations. When such women are involved in sexual relationships with partners without genital herpes, they should be encouraged to be sure that condoms are used for all sexual intercourse. Strategies to reduce subclinical shedding, such as the use of antiviral therapy, need to be developed.

We are indebted to the technologists at the University of Washington Virology Herpes Culture Laboratory, without whom this study could not have been performed, for their skill and dedication.

REFERENCES

- Mertz GJ, Benedetti J, Ashley R, Selke SA, Corey L. Risk factors for the sexual transmission of genital herpes. *Ann Intern Med* 1992;116:197-202.
- Mertz GJ, Schmidt O, Jourden JL, et al. Frequency of acquisition of first-episode genital infection with herpes simplex virus from symptomatic and asymptomatic source contacts. *Sex Transm Dis* 1985;12:33-9.
- Bryson YJ, Dillon M, Bernstein DI, Radolf J, Zakowski P, Garratty E. Risk of acquisition of genital herpes simplex virus type 2 in sex partners of persons with genital herpes: a prospective couple study. *J Infect Dis* 1993;167:942-6.
- Brown ZA, Benedetti J, Ashley R, et al. Neonatal herpes simplex virus infection in relation to asymptomatic maternal infection at the time of labor. *N Engl J Med* 1991;324:1247-52.
- Barton SE, Davis JM, Moss VM, Tyns AS, Munday PE. Asymptomatic shedding and subsequent transmission of genital herpes simplex virus. *Genitourin Med* 1987;63:102-5.
- Brown ZA, Vontver LA, Benedetti J, et al. Genital herpes in pregnancy: risk factors associated with recurrences and asymptomatic viral shedding. *Am J Obstet Gynecol* 1985;153:24-30.
- Rooney JF, Felsler JM, Ostrove JM, Straus SE. Acquisition of genital herpes from an asymptomatic sexual partner. *N Engl J Med* 1986;314:1561-4.
- Brock BV, Selke S, Benedetti J, Douglas JM Jr, Corey L. Frequency of asymptomatic shedding of herpes simplex virus in women with genital herpes. *JAMA* 1990;263:418-20.
- Ashley RL, Militoni J, Lee F, Nahmias A, Corey L. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus type 1 and 2 in human sera. *J Clin Microbiol* 1988;26:662-7.
- Langenberg A, Benedetti J, Jenkins J, Ashley R, Winter C, Corey L. Development of clinically recognizable genital lesions among women previously identified as having "asymptomatic" herpes simplex virus type 2 infection. *Ann Intern Med* 1989;110:882-7.
- Lafferty WE, Krofft S, Remington M, et al. Diagnosis of herpes simplex virus by direct immunofluorescence and viral isolation from samples of external genital lesions in a high-prevalence population. *J Clin Microbiol* 1987;25:323-6.

12. McCullagh P, Nelder JA. Generalized linear models. London: Chapman and Hall, 1983:73.
13. Benedetti J, Corey L, Ashley R. Recurrence rates in genital herpes after symptomatic first-episode infection. *Ann Intern Med* 1994;121:847-54.
14. Koelle DM, Benedetti J, Langenberg A, Corey L. Asymptomatic reactivation of herpes simplex virus in women after the first episode of genital herpes. *Ann Intern Med* 1992;116:433-7.
15. Adam E, Dreesman GE, Kaufman RH, Melnick JL. Asymptomatic virus shedding after herpes genitalis. *Am J Obstet Gynecol* 1980;137:827-30.
16. Adam E, Kaufman RH, Mirkovic RR, Melnick JL. Persistence of virus shedding in asymptomatic women after recovery from herpes genitalis. *Obstet Gynecol* 1979;54:171-3.
17. Stenzel-Poore MP, Hallick LM, Fendrick JL, Neuburg M, Storrs FJ, Hanifin JM. Herpes simplex virus shedding in genital secretions. *Sex Transm Dis* 1987;14:17-22.
18. Barton SE, Wright LK, Link CM, Munday PE. Screening to detect asymptomatic shedding of herpes simplex virus (HSV) in women with recurrent genital HSV infection. *Genitourin Med* 1986;62:181-5.
19. Bowman CA, Woolley PD, Herman S, Clarke J, Kinghorn GR. Asymptomatic herpes simplex virus shedding from the genital tract whilst on suppressive doses of oral acyclovir. *Int J STD AIDS* 1990;1:174-7.
20. Straus SE, Seidlin M, Takiff HE, et al. Effect of oral acyclovir treatment on symptomatic and asymptomatic virus shedding in recurrent genital herpes. *Sex Transm Dis* 1989;16:107-13.
21. Corey L, Adams HG, Brown ZA, Holmes KK. Genital herpes simplex virus infections: clinical manifestations, course, and complications. *Ann Intern Med* 1983;98:958-72.
22. Rattray MC, Corey L, Reeves WC, Vontver LA, Holmes KK. Recurrent genital herpes among women: symptomatic v. asymptomatic viral shedding. *Br J Vener Dis* 1978;54:262-5.
23. Koutsky LA, Stevens CE, Holmes KK, et al. Underdiagnosis of genital herpes by current clinical and viral-isolation procedures. *N Engl J Med* 1992;326:1533-9.
24. Johnson RE, Nahmias AJ, Magder LS, Lee FK, Brooks CA, Snowden CB. A seroepidemiologic survey of the prevalence of herpes simplex virus type 2 infection in the United States. *N Engl J Med* 1990;321:7-12.

Massachusetts Medical Society
Registry on Continuing Medical Education

To obtain information on continuing medical education courses in the New England area, call between 9 a.m. and noon, Monday through Friday, (617) 893-4610 or in Massachusetts 1-800-322-2303, ext. 1342.