Published in final edited form as:

Sex Transm Infect. 2018 December ; 94(8): 568–570. doi:10.1136/sextrans-2017-053244.

A Prospective Cohort Study Showing Persistent HSV-2 Shedding in Women with Genital Herpes 2 Years after Acquisition

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Abstract

Objectives: Herpes simplex virus type 2 (HSV-2) is a prevalent infection with great variability in clinical and virological manifestations among individuals. This prospective cohort study aims to evaluate the natural history of HSV-2 reactivation in the genital area in the same group of women over time.

Methods: Eighteen immunocompetent HSV-2 seropositive women were evaluated for viral shedding for 70 consecutive days within a median of 8 months (range 1–24 months) of HSV-2 acquisition and again approximately two and one half years later from the original study. Participants obtained daily swabs of genital secretions for HSV PCR and recorded genital symptoms.

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Contributions of authors to the manuscript:

SS, ASM, GB, LC and AW designed the study MLH contributed to analysis of specimens. ASM developed the statistical analysis plan and performed the statistical analysis. MR contributed to analysis of the data and writing of the manuscript. All authors contributed to approval of the final manuscript. The authors designed and executed the study, had full access to the raw data, performed all analyses, wrote the manuscript, and had final responsibility for the decision to submit for publication.

Conflicts of Interest:

Conflict of interest: Dr. Anna Wald has received research grants from Genocera and Vical and is a consultant for Aicuris and GSK. Dr. Lawrence Corey is on the scientific advisory board for and holds stock (<1% of the company) in Immune Design Corp, and is a co-inventor listed on several patents involving potential HSV vaccine development. Dr. Amalia Magaret is a consultant for Aicuris and Immune Design. For the remaining authors, none were declared.

Study Approval:

Ethical approval was obtained from the Ethics Committee/Institutional Review Board at University of Washington. Approval Number IRB no. 00001279

Results: The viral shedding rate was 29% during the initial study and 19% in the follow up study (32% reduction, $p=0.019$). Subclinical shedding rate also decreased from 24% to 13% (37% reduction, $p=0.032$), as did the rate of days with genital lesions from 22% to 15% (33% reduction, $p=0.24$). The mean copy number during viral shedding remained unchanged over time at $4.8 \log_{10}$ c/ml (standard deviation = 2.0 and 1.6 during each study, respectively, $p=0.33$). Women with high viral shedding rates in the past were likely to continue to have high shedding rates ($r=0.63$, $p=0.005$).

Conclusions: Despite some reduction, high viral shedding rates persist in women with genital HSV-2 greater than 2 years after acquisition.

Short Summary:

A study of genital herpes simplex virus type 2 shedding in women with genital herpes using a PCR based assay found high viral shedding rates persist years after acquisition.

Keywords

Herpes Simplex Virus Type 2; Genital herpes; viral shedding; natural history

INTRODUCTION:

Herpes simplex virus type 2 (HSV-2) is the leading cause of genital ulcer disease ^[1] with more than 400 million people infected worldwide. HSV-2 infection is a major risk factor for both the acquisition and transmission of HIV-1 ^[2]. The transmission of HSV-2 to partners usually occurs during episodes of asymptomatic shedding, with viral replication but no evidence of clinical disease ^[3]. Counseling of patients regarding transmission of HSV-2 is difficult because of high variability in viral shedding rates among individuals. To assess whether shedding rates change or remain stable in individual persons, we enrolled women who have participated in a prior shedding study ^[4, 5] to obtain genital swabs approximately 2.5 years later.

METHODS:

Study participants and procedures:

The study was conducted at the University of Washington (UW) Virology Research Clinic with HSV-2 seropositive women 2 years of acquisition of genital herpes infection ^[4, 5]. Approximately 2.5 years later, these women returned to clinic to participate in a similar study. Only swabs from women not on antiviral therapy were used for analysis. UW Human Subjects Review Committee approved protocols and participants signed informed consent. After training, women obtained daily swabs of genital secretions of the cervico-vaginal, vulvar and perianal area ^[4]. Participants obtained a separate swab of any genital lesions, maintained a diary recording of genital lesions, and were seen in clinic every two weeks.

Laboratory Methods:

HSV-1 and HSV-2 serostatus was established by the UW Western blot ^[6]. Vulvar and cervical swabs were evaluated by culture and PCR (polymerase chain reaction), while

perianal swabs were evaluated by culture alone due to cost considerations^[5]. Samples were evaluated for HSV DNA by PCR with specimens positive if results ≥ 150 copies of HSV DNA per mL of transport media (c/mL). Data from PCR were used for the reported analyses of vulvar and cervical swabs; findings using viral culture results are included in the supplement.

Definitions and Statistical Analyses:

Days with HSV detected by PCR (or isolated from culture) from any swab sample were considered days with viral shedding. Viral shedding rate was the number of days with positive PCR (or culture) divided by the number of days samples were collected. Subclinical shedding rate was calculated similarly, but excluded days with genital lesions from both numerator and denominator. Recurrences were defined as the presence of any genital lesion consistent with HSV infection noted by the study participant or study clinician. The duration of a lesion was computed as the number of consecutive days with lesion. A Poisson regression in a linear mixed effects model with empirical variance estimation was done to estimate the change in shedding rate, the recurrence rate, and genital lesions over time. Linear mixed models were used to assess changes in quantity of HSV DNA on positive swabs. Correlations between measures over time were also assessed using Spearman's correlation.

RESULTS:

Eighteen of 28 women (64%) who completed the initial study of viral shedding agreed to participate in this study. The median age was 26 (range 22–44). The demographic and clinical characteristics of the 18 women who participated in the follow-up study were not found to differ in age, race, or baseline shedding rates from the 10 who declined (data not shown). All participants had symptoms consistent with primary genital herpes at the time of initial diagnosis. All women were HSV-2 seropositive, HIV and HSV-1 seronegative. The time since primary episode to the initial study was a median of 8 months (range 1–24 months) and the median time between the two sampling periods was 2.4 years (range 1.6–2.8 years).

In the follow up study, the median number of days with swabs collected was 66 (range 45–82). The overall HSV shedding rate was 19% (range 0–71, Table 1). Large variability was noted in the shedding rates detected by PCR among individual women with 10 women shedding on 10% of days, 3 on 10–20% of days, 0 on 20–30% of days and 5 on 30% of days. The subclinical shedding rate was 13% (range 0–66). Nine out of eighteen subjects (50%) had at least one day of genital lesions. The mean percent of days with genital lesions was 15% (range 0–59) with an annualized recurrence rate of 7.5. The mean duration of recurrences was 7.1 days.

Between the 2 study periods, the percentage of days on which HSV was detected decreased from 29% to 19%; a 32% decline (RR=0.68, 95% CI 0.50–0.93, p=0.019). However, the women with high viral shedding rates in the past were likely to continue to have high shedding rates (r=0.63, p=0.005). The mean quantity of viral DNA detected remained

unchanged at 4.8 log₁₀ c/ml ((SD) = 2.0 and 1.6 during each study, respectively, p=0.33). An apparent decline in shedding rates was noted at all anatomic sites sampled.

The subclinical shedding rate decreased from 24% to 13%; a 37% reduction (RR=0.63, 95% CI 0.41–0.96, p=0.032). The mean amount of HSV DNA detected averaged 4.7 log₁₀ c/mL in the initial study and 4.4 log₁₀ c/mL in the follow-up study on days without lesions (SD = 2.1 and 1.5 during each study, respectively, p=0.28).

The percentage of days with lesions demonstrated a trend toward reduction from 22% to 15%; a 33% decrease (RR=0.67, 95% CI 0.34–1.34, p=0.24). Recurrence duration was not found to differ, averaging 7.6 days and 7.1 days (p=0.74). Overall 64 recurrences were reported, 37 in the initial study and 27 in the follow-up study, of which 51 (80%) had a determined length. The remaining 13 recurrences were ongoing at the beginning or end of the observation period. There was a modest correlation in genital lesion rate over time ($r_s=0.39$, p=0.10).

DISCUSSION:

To our knowledge, this study is the first to describe the natural history of viral shedding in the same group of persons at two time periods after acquisition of genital HSV-2 infection. Several measures of viral activity declined over the course of genital herpes, including overall shedding rate, subclinical shedding rate, and lesions. However, many participants continued to have high rates of reactivation of genital HSV-2 almost three years after acquisition, with an average of about 1 out of 5 days with detectable virus in the genital tract.

We found that women with higher shedding rates in the initial study continued to have high shedding rates, supporting the idea of a virologic “set point”, at which the virus-host interaction becomes established, similar to that observed in HIV^[7]. Differences both in the immune response and in HSV-2 strains has been described, but whether host immunity or viral virulence determines the clinical course of HSV-2 infection is unclear^[8].

While a decline in HSV shedding rates appeared consistent among different anatomic sites, the vulvar and perianal areas remained a common site of HSV reactivation, while cervical shedding became less frequent in the follow up study. Potential reasons for the differential decline in shedding rates may include differences in local immunity or differences in the ability of HSV to reactivate efficiently in various types of nerves^[8]. The relative importance of shedding at each anatomic site with regard to infectiousness has not been determined, especially as condoms are likely to be more effective for cervical than for vulvar and perianal shedding.

In contrast to the frequency of shedding, the quantity of HSV, when detected, did not decrease with time. Mathematical modeling of genital HSV-2 has predicted that transmission of virus is more likely to occur at viral loads greater than 10⁴ HSV DNA copies ^[9], and the quantities of HSV observed in this study suggests that the risk of HSV-2 transmission to sexual partners and neonates remains even years after acquisition of this virus.

Strengths of this study include the enrollment of the same participants in the follow-up study to help elucidate the dynamics of intra-person reactivation of HSV-2 over time. There were a small number of participants, but they cooperated with daily sampling which is labor intensive but provides an accurate assessment of shedding rates. The only eligibility criterion for participation was symptomatic acquisition of genital HSV-2 within 2 years of study enrollment, without any severity requirement, thus potentially generalizable to HSV-2 seropositive persons. While not all participants in the first study subsequently enrolled in the follow up study, their demographic and clinical characteristics were similar. It is possible that shedding observed at one site in the genital tract is a result from contamination from a different site but widespread genital reactivation occurs in HSV-2 disease and is associated with a localized HSV-specific T cell infiltrate ^[10].

In summary, this study provides further evidence that genital HSV-2 infection is associated with high shedding rates which decline somewhat with time in most persons. However, high shedding rates tend to persist for several years. Current recommendations for prevention of HSV transmission include disclosure to sex partners, consistent use of condoms and antiviral therapy. Persons with HSV-2 infection should be counseled that even without genital lesions and years after acquisition of infection, they continue to pose a potential risk of viral transmission to sexual partners.

Sources of Financial Support:

This work was supported by grants from the National Institutes of Health: T32 AI007044–38 (MR); P01 AI030731 (AW, SS, AM, LC); K24 AI071113 (AW)

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Table.

Frequency of HSV-2 shedding in the genital tract or perianal area among participants.

	Initial Study	Follow-up Study	
Mean HSV detection by PCR (n/N)			
HSV detection frequency **			
Genital shedding	29% (351/1197)	19% (226/1168)	32% decline (p=0.019)
Cervical shedding	25% (293/1197)	14% (159/1167)	
Vulvar shedding	25% (302/1197)	18% (212/1167)	
Subclinical genital shedding	24% (223/933)	13% (132/1003)	37% decline (p=0.032)
Subclinical cervical shedding	21% (191/933)	9% (85/1002)	
Subclinical vulvar shedding	20% (184/933)	12% (120/1002)	
Quantity in log10 c/mL (range)			
Genital on positive swabs	4.8 (2.2, 9.3)	4.8 (2.2, 8.4)	p=0.33
Genital on positive, non-lesional swabs *	4.7 (2.2, 9.3)	4.4 (2.2, 8.2)	p=0.28
Cervical on positive swabs	4.5 (2.2, 9.3)	4.4 (2.2, 8.2)	
Cervical on positive, non-lesional swabs	4.6 (2.2, 9.3)	4.5 (2.3, 8.2)	
Vulvar on positive swabs	4.7 (2.2, 9.3)	4.6 (2.2, 8.4)	
Vulvar on positive, non-lesional swabs	4.5 (2.2, 9.3)	4.1 (2.2, 7.4)	
Mean HSV isolated by viral culture (n/N)			
HSV isolation frequency **			
Genital shedding	12% (139/1200)	7% (76/1168)	
Cervical shedding	4% (44/1180)	1% (10/1168)	
Vulvar shedding	7% (82/1181)	4% (42/1168)	
Perianal shedding	4% (47/1195)	4% (44/1168)	
Subclinical genital shedding	7% (68/932)	3% (29/1000)	
Subclinical cervical shedding	4% (32/921)	1% (6/1000)	
Subclinical vulvar shedding	3% (26/925)	1% (10/1000)	
Subclinical perianal shedding	2% (20/928)	2% (19/1000)	
Mean Frequency of genital lesions			
Rate of genital Lesion	22% (280/1266)	15% (192/1309)	33% decline (p=0.24)
Total number of recurrences	37	27	
Annualized recurrence rate/year	11.0	7.5	p=0.18
Mean Duration of recurrences, days ***	7.6	7.1	p=0.74

* non-lesional means no lesions at any site on that day

** HSV culture data available for cervical, vulvar and perianal sites. HSV PCR data available from cervical and vulvar sites.

*** 51 (80%) recurrences were available to determine length. The remaining 13 recurrences were ongoing at the beginning or end of the observation period.

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