

## Herpes Simplex Virus Viremia during Primary Genital Infection

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**Primary genital herpes simplex virus (HSV) infection is commonly associated with systemic symptoms. A systematic evaluation of HSV viremia during primary genital infection has not been performed previously. Plasma samples from adults with a first clinical episode of genital HSV infection were assayed for HSV DNA by polymerase chain reaction. One hundred sixty-four adults had confirmed primary genital HSV infection. Of these, 40 (24%) had HSV DNA detected in plasma. Thirty-seven (93%) of 40 were infected with HSV-2. Viremic participants were more likely to be women, compared with aviremic participants (83% vs. 61%;  $P = .01$ ). Viremia was detected more frequently and at a higher number of copies per milliliter early in infection. We conclude that HSV viremia during primary genital HSV infection is common, especially among women.**

The clinical manifestations of primary genital herpes simplex virus (HSV) infection frequently include systemic and meningeal symptoms [1]. The pathogenesis of initial genital HSV in-

fection is poorly characterized, as patients usually present several days after the appearance of genital symptoms. Infection of extragenital sites that lie outside of sacral ganglia innervation can occur either during primary infection or during recurrences, suggesting complex mechanisms for broad viral spread [1, 2]. Potential explanations for both systemic symptoms and extragenital disease include dissemination of the virus through the blood (viremia), spread of infection between ganglia [3], or cutaneous inoculation at the extragenital site of infection. Studies of HSV infection in the guinea pig model have demonstrated that HSV-2 rapidly disseminates after primary genital infection, reaching the dorsal root ganglia within 2 days and the brain stem and cerebral cortex by day 5 [4]. Animal models of infection have not detected HSV-2 in the blood.

The ability to detect HSV DNA by polymerase chain reaction (PCR) in samples from mucosal surfaces, spinal fluid, and blood has changed our understanding of the natural history of the disease [5]. PCR is a highly sensitive technique, which allows more frequent detection of virus, compared with viral culture [6]. While HSV viremia has been demonstrated in infants [7] and adults with disseminated disease [8], systematic evaluation of HSV viremia during primary genital HSV infection by use of PCR has not been previously performed. We present the first large cohort study of HSV viremia among an immunocompetent population with primary genital herpes.

**Materials and methods.** Participants with a first clinical episode of genital herpes were recruited through the University of Washington Virology Research Clinic. Between 1974–1981 and 1995–2002, a total of 2 cohorts (331 patients) with a first clinical episode of genital herpes participated in studies of genital HSV disease [1]. Of 331 participants with first episode genital HSV disease, 180 had plasma samples available for PCR and were included in this study. Primary genital herpes was defined as an HSV-1 or HSV-2–negative serological result at the initial visit and an HSV-positive culture of samples from the genital lesion or HSV seroconversion documented by Western blot analysis [1]. Participants provided demographic information, health history, and symptom review at the first visit. The earlier cohort was followed up every other day until resolution of the episode. The later cohort was seen once during initial infection. Participants in both cohorts were followed up for recurrences. Written, informed consent was obtained from each participant and the studies were approved by the University of Washington institutional review board.

Blood samples were collected at initial and follow-up visits, with up to 7 plasma samples collected from participants in the

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**Table 1. Demographic and clinical characteristics of 164 individuals with primary genital herpes, by viremic status and cohort membership.**

Characteristic	Overall	Viremia status		P	Cohort membership		P
		Viremic	Aviremic		Early <sup>a</sup>	Late <sup>b</sup>	
Female sex	108/164 (66)	33/40 (83)	75/124 (61)	.01	76/111 (69)	32/53 (60)	.31
White race	146/162 (90)	40/40 (100)	106/122 (87)	.01	106/109 (97)	40/53 (76)	.001
Age, median (range), years	25 (16–57)	24 (17–38)	25 (16–57)	.21	25 (16–52)	25 (17–57)	.24
Initial episode							
Primary HSV-1 infection	43/164 (26)	3/40 (8)	40/124 (32)	...	17/111 (15)	26/53 (49)	...
Primary HSV-2 infection	121/164 (74)	37/40 (92)	84/124 (68)	.002 <sup>c</sup>	94/111 (85)	27/53 (51)	<.001 <sup>c</sup>
Culture positive for HSV	129/156 (83)	37/40 (93)	92/116 (79)	.06	89/110 (81)	40/46 (87)	.36
Duration of outbreak, mean ± SD, days	19 ± 8	21 ± 9	18 ± 8	.19	21 ± 9	15 ± 5	<.001
Days after onset of symptoms, median no. (range)	4 (0–25)	4 (0–15)	4 (0–25)	.36	5 (0–25)	4 (0–18)	.2
Sexual history							
Monogamous during past month	108/140 (77)	25/36 (69)	83/104 (80)	.20	63/87 (72)	45/53 (85)	.09
Age of sexual debut, median (range), years	17 (11–27)	17 (12–23)	17 (11–27)	.31	18 (12–23)	18 (11–27)	.09
Prior oral ACV treatment	52/164 (32)	12/40 (30)	40/124 (32)	.79	18/111 (16)	34/53 (64)	<.001

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. HSV, herpes simplex virus; ACV, acyclovir.

<sup>a</sup> Data obtained between 1974–1981.

<sup>b</sup> Data obtained between 1995–2002.

<sup>c</sup> For comparison between primary HSV-2 infection and HSV-1 infection.

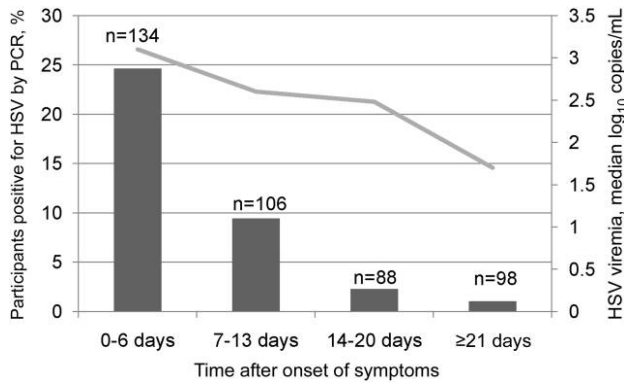
early cohort. Samples were collected from genital lesions with a Dacron swab and placed in viral transport media for HSV culture. All samples were stored at  $-20^{\circ}\text{C}$  until the time of analysis.

HSV culture was performed on samples obtained from genital lesions, as described elsewhere [1]. Acute phase serum samples were collected at the initial visit and tested for HSV-1 and HSV-2 antibodies with Western Blot analysis [9]. HSV DNA was amplified from plasma by a real-time fluorescence based PCR, as described elsewhere [6]. Viremia was defined as  $\geq 50$  copies HSV DNA per mL of plasma [10]. Among the 12 HSV-negative plasma controls that were included per 96-well plate, no contaminants were detected. To test for encapsulated HSV DNA in plasma, selected plasma samples were treated with DNase, as described elsewhere [11]. To confirm that DNase treatment was effective, samples were confirmed negative for varicella-zoster virus (VZV) DNA by PCR, and then spiked with  $5 \times 10^4$  copies of purified VZV DNA. The samples were divided into 2 equal aliquots. One aliquot was treated with DNase, and the other was not. PCR analyses for HSV and VZV were then performed in parallel.

Participants who had HSV-1 antibody detected at the initial visit were not considered to have primary genital HSV infection and therefore were excluded from statistical analysis. Viremic participants were defined as those who had HSV DNA detected in any available plasma samples within 28 days after onset of initial symptoms. Characteristics of participants with and without viremia were compared using the Wilcoxon rank sum test for nonparametric continuous data and the Student's *t* test for continuous, normally distributed data. For binary variables, the  $\chi^2$  test was performed to determine the difference in the rates of

symptoms on the basis of exposure to viremia. Participants in the later cohort did not record symptoms at the initial visit. Therefore, the symptom data in the study reflect only those participants in the earlier cohort. We performed univariate and multivariate relative risk regression (adjusting for age, sex, and race) using a generalized linear model with Poisson distribution and robust standard error estimates to determine associations between viremia and symptoms. The time to first recurrence was analyzed by use of the Kaplan-Meier survival method and compared between groups by use of the log rank test. Negative binomial regression was used to calculate the difference in recurrence rate (i.e., the number of documented recurrences over time) between viremic and aviremic individuals. A 2-sided *P* value  $< .05$  was considered statistically significant. Statistical calculations were performed with Intercooled Stata (version 9.1; Stata).

**Results.** Of 180 participants with initial genital herpes infection, 16 (9%) had evidence of prior HSV-1 infection and were excluded from further analysis. Of the 164 participants with primary genital herpes, 108 (66%) were women and 146 (90%) were white (table 1). A total of 121 participants (74%) were infected with HSV-2 and 43 (26%) were infected with HSV-1. HSV DNA was detected in plasma from 40 (24%) of 164 participants and in 46 (11%) of the 426 plasma samples analyzed. The frequency of viremia was significantly higher among participants infected with HSV-2, compared with participants infected with HSV-1 (31% vs. 7%; *P* = .002). The median age did not differ significantly between viremic and aviremic participants (24 vs. 25 years). A significantly larger proportion of viremic partici-



**Figure 1.** Proportion of plasma samples showing herpes simplex virus (HSV) viremia, relative to time after onset of initial symptoms of primary HSV infection. *Gray line*, amount of HSV DNA detected in plasma samples over the 4-week period. Counts are the total no. of plasma samples collected during each period.

pants were women, compared with aviremic participants (83% vs. 61%;  $P = .01$ ).

White race was also associated with viremia (100% of viremic participants vs. 87% of aviremic participants;  $P = .01$ ). Individuals with viremia tended to be more likely to have a culture of genital lesion samples that was positive for HSV, compared to participants without viremia (93% vs. 79%;  $P = .06$ ). Most participants (88%) were seen within 10 days after the onset of symptoms, and there was no difference in time to presentation for viremic participants, compared with aviremic participants (median, 4 days). Viremia was not associated with longer duration of the primary episode (mean, 18 vs. 21 days;  $P = .19$ ).

The early cohort was less racially diverse than the later cohort (97% of participants in the early cohort were white vs. 76% of participants in the later cohort;  $P = .001$ ), but the cohorts were otherwise similar with respect to demographic and clinical characteristics. However, members of the early cohort were more likely to have primary genital infection caused by HSV-2, compared with members of the later cohort (85% vs. 51%;  $P < .001$ ). In addition, significantly fewer participants in the early cohort had received oral acyclovir at the time of study enrollment, compared with participants in the later cohort (16% vs. 66%;  $P < .001$ ). Although a greater proportion of participants in the early cohort tended to be viremic, compared with the later cohort (29% vs. 15%;  $P = .06$ ), this difference did not remain after adjusting for HSV-2 infection.

HSV viremia was found most frequently during the first week after onset of initial symptoms of primary genital herpes (figure 1). A total of 33 (25%) of 134 plasma samples collected during the first 7 days after primary infection were positive for HSV DNA. In the second, third, and fourth weeks of infection, the number of plasma samples containing HSV DNA decreased to 10 (9%) of 106, 2 (2%) of 88, and 1 (1%) of 98, respectively. The

amount of HSV DNA detected in plasma samples followed a similar trend, with median 3.1 log<sub>10</sub> copies/mL HSV DNA detected in the first week of infection, and 1.7 log<sub>10</sub> copies/mL detected in the fourth week of infection. The number of copies of HSV DNA in plasma ranged from 1.0–6.0 log<sub>10</sub> copies/mL, with a median copy number of 3.0 log<sub>10</sub> copies/mL. No differences in demographic or clinical characteristics were found between participants with a high number of copies ( $\geq 4.0$  log<sub>10</sub> copies/mL), compared with those who had a low number of copies.

To evaluate whether plasma HSV DNA was encapsulated within virions, we selected 6 plasma samples with a high number of copies of HSV DNA from different participants and compared the HSV DNA copy number before and after treatment with DNase. HSV DNA was detected after DNase treatment in 4 of the 6 samples. The median number of copies before and after DNase treatment was 3.5 log<sub>10</sub> copies/mL versus 2.5 log<sub>10</sub> copies/mL, respectively. Purified VZV DNA was spiked into each sample and was detected in all samples before DNase treatment, but in none of the samples after DNase treatment. These data suggest that much of the HSV DNA detected in plasma was encapsulated.

To determine whether HSV viremia during primary infection was associated with systemic symptoms, we analyzed the symptoms reported by participants at the time of presentation. Several systemic and meningeal symptoms were significantly associated with viremia, including fever (prevalence risk ratio [RR], 2.73;  $P < .001$ ), constitutional symptoms, (RR, 1.74;  $P < .001$ ), headache (RR, 1.54;  $P = .02$ ), stiff neck (RR, 1.7;  $P = .01$ ), and photophobia (RR, 2.06;  $P = .02$ ). Localized symptoms, such as pain and itching, were not associated with viremia. However, dysuria (RR, 1.52;  $P < .001$ ) and vaginal discharge (RR, 1.40;  $P = .04$ ) were associated with viremia. Fever and dysuria remained significantly associated with viremia after adjustment for age, sex, and race.

Compared with aviremia, viremia was associated with a somewhat shorter median time to first recurrence (64 vs. 43 days;  $P = .35$ ) and a higher subsequent rate of genital recurrences (incidence rate ratio, 1.30;  $P = .20$ ), but these findings were not statistically significant.

**Discussion.** HSV viremia was observed in 24% of immunocompetent adults with primary genital infection, providing insight into HSV pathogenesis and the clinical manifestations of HSV mucocutaneous disease. HSV viremia was especially common among women, compared with men, and among participants infected with HSV-2, compared with those infected with HSV-1. The presence of HSV in the blood compartment has been previously described in several clinical settings, including neonatal infection [7] and immunocompetent adults with disseminated disease [8]. To our knowledge, however, this is the first study to demonstrate that persons with localized genital disease frequently have detectable levels of HSV DNA in plasma. Plasma HSV was resistant to treatment with DNase, which supports the hypothesis that the HSV DNA is encapsulated within

infectious virions. Although isolation of HSV by culture would have provided additional evidence of infectious virus in plasma, our samples were not stored at the proper temperature to perform this experiment. Moreover, studies of HSV meningitis [3], encephalitis [12], and mucosal infections [13] have shown the clinical relevance and transmissibility of virus in samples that are positive for HSV by PCR but negative by culture.

The increased frequency of HSV detection in plasma samples from women, compared with men, is consistent with the clinical findings that systemic symptoms and infection at extragenital sites are more common in women [1]. For instance, recurrent benign lymphocytic meningitis is a disease that is seen predominantly in women [3]. The pathogenesis of this disease has previously been hypothesized to be retrograde seeding of the central nervous system from the sacral ganglion [3]. As plasma may potentially serve as a source of new foci of infection at nongenital sites, viremia during primary genital infection provides another mechanism for the localization of virus to cerebrospinal fluid. In animal models, it has been shown that after intravenous infection with HSV, the virus selectively establishes latency in both central and peripheral neural tissue [14].

We hypothesized that participants with viremia would have more frequent genital recurrences due to a higher burden of virus establishing latency in neuronal ganglia. The quantity of latency-associated transcripts is correlated with recurrence rates in guinea pigs infected with HSV-1 and HSV-2 [15], which suggests that there is a relationship between the quantity of virus establishing latency and recurrent disease. We observed a trend toward a 30% increase in recurrence and decreased time to first recurrence in viremic participants, compared with aviremic participants, although our study was underpowered to definitively answer this question.

This study demonstrated that HSV viremia is common during primary genital infection, especially in women, and is associated with systemic symptoms. Larger prospective studies of primary genital herpes will be required to definitely determine the effects of viremia during primary infection on the natural history of genital herpes.

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