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Oral shedding of herpes simplex virus type 1 in immunocompetent persons

Stanley C. Gilbert

Department of Medicine, University of Washington, Seattle WA 98195, USA; Dermatology and Laser Center NW, Bellingham, WA 98225, USA

BACKGROUND: Reactivation of herpes simplex virus-1 (HSV-1) can result in recurrent herpes labialis lesions (RHL) and in oral shedding of virus. This study utilized polymerase chain reaction (PCR) to document the frequency and quantity of such shedding.
METHODS: Thirty adults with greater than three RHL episodes per year were followed through one recurrence. They collected swabs for PCR every 12 h starting at prodrome and for 10 days thereafter. Shedding was analyzed with regard to frequency, timing and quantity.
RESULTS: HSV-1 was detectable in 87% of participants for a mean of 4 days. Shedding occurred most frequently during the vesicle/ulcer stage (91% of subjects), but was common in both clinical and subclinical stages (50% vs. 23%, average log DNA copy number/ml^2 2.6 vs. 1.4).
CONCLUSION: The majority of RHL patients shed viral DNA. Shedding occurred before and after the appearance of clinical lesions. Such findings may be useful in designing methods to reduce viral shedding and prevent transmission.
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Keywords: cold sore; herpes labialis; herpes simplex virus-1; polymerase chain reaction; oral shedding; subclinical shedding

Introduction

Approximately 60%–80% of the adult population in the United States is chronically infected with herpes simplex virus (HSV)-1. Among those infected, 20%–40% experience periodic reactivation of the virus leading to recurrent herpes labialis lesions (RHL) or asymptomatic shedding (1–5). Few data exist to document the frequency, quantity and duration of oral HSV-1 shedding. Oral viral shedding is important because of its suspected role in transmission of oral, genital or cutaneous infections (1, 2, 6–8). This study was conducted to measure HSV-1 shedding with PCR to gain more insight into how HSV-1 is shed during outbreaks.

Prior studies that documented oral shedding of HSV-1 with RHL have used viral cultures and have followed small numbers of participants (9–13). However, viral culture is an insensitive method for detecting HSV on mucosal and cutaneous surfaces compared with the polymerase chain reaction (PCR) assay (14–18), and recent studies of genital and oral HSV shedding have utilized PCR. In this study, we evaluated daily oral shedding of HSV around the time of a RHL lesion using PCR.

Materials and methods

Study participants and procedures

The data reported in this paper are from a double-blind, randomized study that compared oral shedding rates of HSV-1 between a placebo and valacyclovir treatment arm. Because of the lack of published PCR-based shedding data regarding the natural history of herpes labialis, this report focuses exclusively on the results of the placebo arm of this trial. Thirty-four healthy immunocompetent men and women were enrolled in the placebo arm: they were at least 18 years of age, had a history of three or more episodes of RHL per year, with at least 50% of the recurrences preceded by prodromal symptoms (e.g. localized numbness, tingling, and pain). At the first sign of prodrome, the participants were instructed to vigorously swab the lips and orolabial mucosal surfaces with a Dacron swab. The swabs were placed in PCR buffer and refrigerated. The swabbing procedure was repeated twice daily for the next 10 days. Participants used diaries to record the date and time of the onset of prodrome (defined as 'day 1'), symptoms, and lesion stage (prodrome, macule, papule, vesicle/ulcer, crust, healed) at the time of each swabbing. Participants were seen by research staff within 24 h of their first prodromal symptoms, then at days 5 and 10 to assess lesion healing. An oral examination was performed at each visit for RHL, diaries were reviewed and swabs were collected. No systemic or topical antivirals

Correspondence: Stanley C. Gilbert MD, Dermatology and Laser Center Northwest, 3614 Meridian, Suite 200, Bellingham, WA 98225, USA. Tel.: +1 360 676 4205. Fax: +1 360 676 0377. E-mail: scgilbert@hinet.org
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were administered during this time. The research was approved by the Western IRB and all participants gave written consent.

Laboratory procedures
A real-time fluorescent probe-based PCR (Taqman; Applied Biosystems, Foster City, CA, USA) method was used to detect and quantitate HSV in the swab samples in both studies (19). The optimized gB forward primer used was CCG TCA GCA CCT TCA TCG A, the reverse primer was CGC TGG ACC TCC GTG TAG TC and the probe was CCA CGA GAT CAA GGA CAG CGG CC.

Statistical methods
The objective of the study was to quantify HSV-1 shedding associated with a lesion of RHL from the onset of prodrome and for 10 days thereafter. The primary endpoint was the proportion of the 10-day period with viral shedding. A secondary endpoint was to compare shedding during stages when lesions were clinically evident ('clinical stages') vs. those in which they were not ('subclinical stages'). Summary measures included: (i) the percentage of subjects reporting each lesion stage, (ii) the percentage of subjects with shedding during the 10-day period and during each stage, (iii) the mean time in each stage, (iv) the mean time with shedding during the 10-day period and in each stage, (v) the mean percentage of time with shedding during the 10-day period and in each stage, and (vi) the median time to lesion healing. Quantitative measures of HSV-1 viral counts included mean average log DNA copy number (average log copy number was calculated for each subject, then a mean of all subjects was calculated) reported during the 10-day period and during each stage.

Time in a given lesion stage was calculated by counting the number of 12-h diary entries at which the subject reported the given stage and converting to days by multiplying by 0.5 days. Time in stage with shedding was calculated by counting the number of 12-h diary periods at which the subject reported the given stage and had a positive PCR evaluation, then converting this to days. Percentage of stage with shedding was calculated for each subject as time in stage with shedding divided by time in stage $\times 100\%$.

The stages were also aggregated into clinical stages (macule, papule, vesicle/ulcer, crust) and subclinical stages (prodrome, healed). Summaries included the percentage of time in the clinical and subclinical stages with viral shedding as well as the mean of each subject's average log DNA copy number.

Time to lesion healing was calculated as the time from the first report of a clinical stage until the first report of healing. For the purpose of describing PCR shedding during symptomatic RHL, only those subjects who completed all study procedures were analyzed.

Missing stage data were imputed where possible by carrying forward the last non-missing value (last observation carried forward method, LOCF). No such imputations were made for missing DNA copy number data. If the PCR evaluation was negative then the log DNA copy number was taken to be 0.

Continuous endpoints, such as age, number of RHL episodes per year, time with shedding, and average log DNA copy number were summarized with the mean, standard deviation, median, and range. Discrete endpoints such as gender and HSV-1 status were summarized with frequencies and percentages.

An average log DNA copy number was calculated for each subject in each stage. The mean of *all* subjects in that stage was then calculated and referred to as the 'mean average log DNA copy number' for a given stage. As some subjects had isolated days on which no DNA was detected despite PCR evidence of shedding immediately prior to, and following these days, copy numbers were calculated in two different ways. In the first, the copy numbers were averaged only from days on which shedding was detected in a given stage. In the second, copy numbers were averaged over all days in a given stage.

Results

Thirty-four participants (68% female, 32% male) with a median age of 43 (19–79) years were enrolled. The median duration of oral herpes was 30 years (range 4–70) with a median of four (3–12) episodes per year (Table 1). Thirty participants completed all study procedures, and four attended only one clinic visit then discontinued the study without collecting any specimens. Overall, the 30 participants who performed swabbing collected 590 swabs for a 98.3% adherence to the study protocol. Of the 590 swabs collected for detection of viral shedding, 241 (41%) had HSV-1 present. HSV-1 was detected at least once in 26 of 30 (87%) participants during the 10-day sampling period. The mean duration of viral shedding was 4.1 days with a range of 0–9.5 days.

Viral DNA shedding was found at every lesion stage, but was most commonly detected in the vesicle/ulcer stage (Fig. 1). HSV-1 was detected on 80% of the days during this stage. Furthermore, 20 of 22 (91%) subjects who developed the vesicle/ulcer stage had PCR evidence of HSV-1 DNA.

The average log DNA copy number followed a similar trend throughout the stages of lesion develop-

Table 1 Demographic and clinical characteristics of the study participants

Variable	*(n = 34)*
Age	
Median (range)	42.5 (19–79)
Sex, *n* (%)	
Men	11 (32%)
Women	23 (68%)
Ethnic origin, *n* (%)	
Caucasian	34 (100%)
Length of history of recurrent oral herpes (years)	
Median (range)	30 (4–70)
Recurrences in past 12 months	
Median (range)	4 (3–12)

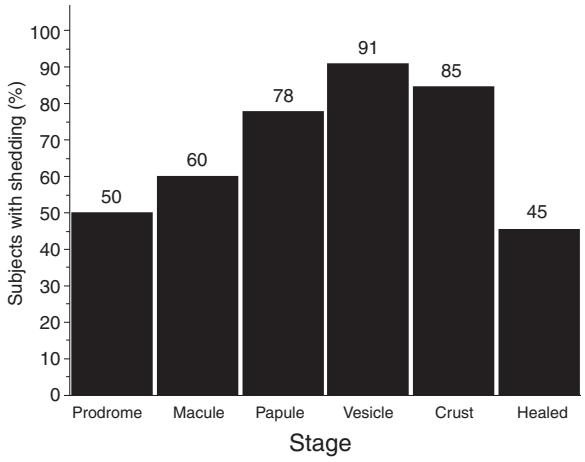

Figure 1 Percent of subjects with shedding by stage.

ment whether it was calculated from days with shedding only or as an average over all days in a given stage. Values peaked at the vesicle /ulcer stage and declined thereafter. The difference in copy numbers between stages was larger when values were averaged over all days in a given stage (Table 2).

To further examine the relationship between viral shedding and the stages of RHL lesions, average DNA copy numbers were analyzed according to the presence or absence of clinical lesions. Stages in which a lesion was clinically evident (macule, papule, vesicle/ulcer and crust) were grouped together as 'clinical' stages, and those in which no active lesion was noted (prodrome, healed) were grouped as 'subclinical.' Shedding occurred on 50% of the days in clinical stages and on 23% of those in subclinical (Table 3). When calculated from shedding days only, average log DNA copy numbers were similar between clinical stages (5.0, SD 0.9) and subclinical stages (5.1, SD 1.2). When calculated as an average of *all* days in stages, average log DNA copy numbers were 2.6 (SD 1.9) vs. 1.4 (SD 1.7).

Table 3 Summary of shedding by clinical and subclinical stages

Stage[a]	*Overall (n = 30)*	*Clinical stages (n = 30)*	*Subclinical stages (n = 28)*
Percentage of stage with shedding[b]			
Mean (SD)	42 (25.6)	50 (31.1)	23 (28.0)
Median	40	50	17
Range	0–95	0–100	0–100
Average log DNA copy number/ml^2 only on days with shedding[c]			
Mean (SD)	5.0 (0.8)	5.0 (0.9)	5.1 (1.2)
Median	5.0	5.0	4.7
Range	3.3–6.7	3.3–7.1	3.0–7.1
Average log DNA copy number/ml^{2}over all days[d]			
Mean (SD)	2.2 (1.5)	2.6 (1.9)	1.4 (1.7)
Median	1.9	2.4	1.0
Range	0.0–5.4	0.0–7.1	0.0–6.4

[a]Missing stage replaced with last observation carried forward.
[b]Based on subjects who reported the stage.
[c]Based on participants who reported the stage and shed virus.
[d]Days with no shedding are imputed as a log DNA copy number = 0.

Discussion

This study shows that shedding of HSV-1 in oral secretions associated with RHL is frequent and occurs commonly during clinical and subclinical stages of lesion development. We used PCR from orolabial sites to document the frequency, duration and quantity of viral shedding. During a RHL episode, twice-daily oral swabs demonstrated viral DNA beginning as early as the onset of prodrome, and throughout all stages of lesion development for up to 10 days (the maximum duration for which samples were collected). Viral shedding was found in 50% of participants at the onset of prodrome, in 91% during the vesicle/ulcer stage, and still as high as 45% during the days on which the lesions were reported as 'healed.' Average log viral DNA copy numbers peaked during the vesicle/ulcer stage, and were higher during clinical vs. subclinical stages when shedding was averaged over all days.

Approximately one-third of HSV-1 infected persons experience symptomatic RHL, and viral shedding by

Table 2 Frequency of HSV-1 shedding by stage of oral herpes lesions

Lesion stage[a]	*Prodrome*	*Macule*	*Papule*	*Vesicle/ulcer*	*Crust*	*Healed*
Percentage of stage with shedding[b]						
Mean	50	56	69	80	45	16
Median (Range)	45 (0–100)	100 (0–100)	100 (0–100)	100 (0–100)	37.5 (0–100)	0 (0–75)
Percent of subjects with shedding during stage[b] *n*/N (%)	10/20 (50)	9/15 (60)	14/18 (78)	20/22 (91)	22/26 (85)	10/22 (45)
Average log DNA copy number/ml^2 during stage only on days with shedding[b]						
Mean	5.3	5.0	5.4	5.7	4.7	4.4
Median (Range)	5.1 (3.4–7.1)	5.0 (3.9–6.3)	5.2 (2.8–7.7)	6.1 (3.5–7.8)	4.4 (2.8–7.1)	4.2 (2.9–3.8)
Average log DNA copy number/ml^2 during stage over all days[c]						
Mean	2.6	2.8	3.7	4.6	2.3	0.7
Median (Range)	1.7 (0.0–7.1)	3.9 (0.0–6.3)	4.6 (0.0–7.1)	5.2 (0.0–7.8)	1.7 (0.0–7.1)	0.0 (0.0–3.8)

[a]Missing stage replaced with last observation carried forward (LOCF). There were three participants with a total of five diary entries for which stages were not reported and therefore could not be imputed with LOCF.
[b]Based on participants who reported the stage and shed virus.
[c]Days with no shedding are imputed as a log DNA copy number = 0.

culture has previously been reported during and immediately following such recurrences (9–13). Viral shedding has also been reported before and after aborted recurrences of oral herpes ('false prodromes') (12), and during intraoral (and often undetected) recurrences of HSV (10). The results of our study may actually underrepresent the true incidence of subclinical shedding as we did not follow subjects with daily PCR sampling over time in between clinically evident recurrences.

In 1953, Buddingh (20) was the first to report HSV isolated from the oral secretions of five of 185 (2.4%) asymptomatic seropositive adults. The rate of shedding was higher (20%) among children aged 7 months to 2 years diagnosed with primary herpetic gingivostomatitis. Of interest, HSV was also isolated from rectal swabs of these same children 3–4 days after it had appeared in saliva. A prospective study of eight adults with a history of RHL who obtained daily oral rinses for 5 months found that HSV-1 could be isolated in 47 of 637 (7.4%) samples (12). It has been found that viral shedding tends to occur in clusters of days [29 of 47 specimens (62%)], and is associated with the common cold (21%), after oral trauma (17%) or in association with a recurrence of herpes labialis (19%). Of particular interest is the finding that the highest rate of shedding (60%) has been found among subjects reporting a false prodrome, that is, persons without a visible recurrence. Additional studies that used viral culture reported shedding on 0.1%–9.0% of days studied (9, 21–24). Krone et al. (25) followed nine men who were seropositive for HSV-1 and HSV-2 for 100 days; daily swabs for viral culture were obtained from oral and genital areas. The participants were seronegative for human immunodeficiency virus. Over the course of the 100 days, HSV-1 was detected by culture on 2.1% of days, and 23 of the 24 positive cultures were obtained from oral, not genital secretions.

More recent PCR-based studies have also demonstrated oral HSV shedding. In a cross-sectional survey of 1000 outpatients attending a Japanese maxillofacial surgical clinic for the first time, HSV-1 was isolated in culture from saliva specimens in 27 persons (2.7%) and HSV-1 was detected by PCR in (4.7%) (26). Among subjects known to be chronically infected with HSV-1, Scott et al. (27) used thermography to confirm the prodromal stage of herpes labialis and showed that HSV-1 could be demonstrated in 45% of such subjects. Kriesel et al. (28) studied HSV -1 shedding in 12 subjects with ultraviolet light induced RHL. HSV-1 shedding was detected by PCR in eight of 12 subjects (67%) in at least one oral rinse specimen. da Silva et al. (29) randomly sampled a group 14 individuals with a history of RHL for oral shedding of HSV-1 DNA by PCR over 11 months. These results were compared with those of 11 seropositive subjects with no history of cold sores. At least one instance of HSV shedding was detected in all individuals regardless of clinical status, and there was no difference between the groups with respect to the percentage of positive samples acquired.

Oral shedding of HSV-1 may provide an opportunity for transmission of this neurotropic virus that must gain access to its host via mucocutaneous barriers. Unlike some other viruses (e.g. human immunodeficiency virus), with HSV-1 there is no defined threshold (viral titer) at which viral transmission is known to occur. It has been presumed that transmission of most cases of HSV-1 occurs via contact with oral secretions, and that primary infections present asymptomatically or with signs and symptoms specific to the site of entry (e.g. gingivostomatitis, herpes gladiatorum, and genital herpes). If the portal of entry happens to be the oral mucosa, HSV-1 establishes permanent residence (latency) in the trigeminal ganglion. During periods of reactivation the virus replicates initially at the sensory ganglion and then spreads in an anterograde fashion along sensory neurons. Thus, viral DNA spreads back to the mucocutaneous domain of the trigeminal nerve (facial skin, ocular and oral mucous membranes). In societies where individuals live in crowded conditions, there are many opportunities for childhood contact with HSV-1-infected oral secretions and, as a result, the majority of the population has been infected with oral HSV-1 by young adulthood.

Although RHL may be considered by many to be a nuisance disease, there are situations in which HSV-1 shedding may lead to serious consequences. In the last decade it has been found that an increasing number of initial episodes of genital herpes are caused by HSV-1, and in some regions HSV-1 actually exceeds HSV-2 as the cause (15, 30–34). It has been hypothesized that delay in the time to acquisition of HSV-1 among populations in the developed world occurs as a result of a rise in living standards and a trend toward more isolated family units (35). Therefore, many individuals are acquiring HSV-1 not from childhood playmates or from the kisses of adult caretakers, but from the oral secretions of their early sexual partners. Instructions to avoid orogenital contact for at least 10 days following a cold sore in the partner providing oral sex, may be useful in reducing the risk of transmission of genital HSV-1.

Another serious consequence of HSV-1 transmission is neonatal herpes. In most cases the transmission occurs from a mother with primary genital herpes who is shedding virus at the time of labor and delivery. In addition, neonates may acquire devastating generalized infections from the HSV-1 shed in the oral or cutaneous secretions of their parents or caretakers (36–46).

Children with atopic dermatitis are also at risk for disseminated cutaneous herpetic infection (eczema herpeticum) acquired from the HSV-1 shed within the oral secretions of their caregivers and playmates (47, 48). In other cases, autoinoculation from a patient's own oral secretions has led to eczema herpeticum (49).

In this report, we have demonstrated that recurrences of herpes labialis are accompanied by the shedding of HSV-1 that may be detectable by PCR from the orolabial mucosa starting before any visible RHL lesions appear, and can occur for up to 10 days and possibly longer. Future studies should include a larger number of subjects, and should include seropositive participants without a history of frequent cold sores. It

would also be of interest to study the impact of suppressive antiviral therapy on shedding and transmission rates of HSV-1.

Conflict of interest

Dr Gilbert has served as a consultant to GlaxoSmith-Kline Pharmaceuticals, Novartis Pharmaceuticals, and Biogen Pharmacetuicals.

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