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Antiretroviral therapy is not associated with reduced herpes simplex virus shedding in HIV coinfected adults: an observational cohort study

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ABSTRACT

Objectives: Herpes simplex virus types 1 and 2 (HSV-1/2) may have adverse consequences on HIV type 1 infection. We quantified the frequency of HSV reactivations in highly active antiretroviral therapy (HAART)-treated adults with HIV, and compared it with that in HAART-naïve patients.

Setting: 2 academic hospital sites in Toronto, Canada.

Participants: Asymptomatic HAART-naive (n=44) or treated (with HIV RNA <50 copies/mL, n=41) adults with HSV-1 and/or 2, HIV coinfection.

Outcome measures: HSV-1 and HSV-2 shedding as measured by PCR on oral, genital and anal swabs self-collected daily for 28 days.

Results: Of the 85 participants, 88%, 67% and 53% were coinfected with HSV-1, HSV-2 and both HSV types, respectively. Median (IQR) CD4 count was 516 (382, 655) cells/mm^3. HSV (type 1 and/or 2) shedding occurred on a median (IQR) of 7.1% (0, 17.9%) of days in HAART users and 3.6% (0, 10.7%) of days in non-HAART users. No significant relationship was observed between HAART and HSV-1/2 shedding in univariable (OR=1.55, 95% CI 0.83 to 2.87) or multivariable negative binomial models adjusted for sex, baseline CD4 count, recent immigrant status and time since HIV diagnosis (adjusted OR, aOR=1.05, 95% CI 0.43 to 2.58). Similar null results were observed for HSV-2 shedding in HSV-2 seropositive participants (aOR=1.16, 95% CI 0.40 to 3.36) and HSV-1 shedding in HSV-1 seropositive participants (aOR=0.70, 95% CI 0.14 to 3.47).

Conclusions: HSV reactivations persist despite suppressive HAART among adults coinfected with HSV and HIV. Clinical trials of suppressive anti-HSV therapy are warranted in this population.

Strengths and limitations of this study

- One of the very few studies to examine subclinical herpes simplex virus (HSV) shedding in adults coinfected with HIV on highly active antiretroviral therapy (HAART).
- Unique in restricting the HAART-treated group to patients with undetectable HIV viral loads, thus maximising its relevance to the modern era.
- Sampling methods may not have captured all anatomic reactivations of HSV infection.
- The enrolment criterion of minimal HSV symptoms may have altered the spectrum of patients included in favour of those with less HSV shedding, although our goal was to document the extent of residual HSV shedding in the least symptomatic HAART-treated persons.
- Study termination at the time of a clinical outbreak may have produced lower HSV shedding rates in those with more active HSV, but was uncommon and occurred in both study groups (3 naïve and 1 HAART).

INTRODUCTION

Coinfection with herpes simplex virus type 2 (HSV-2) is common in HIV infection, and has been associated with increased plasma and genital tract HIV RNA levels,[1 2] increased immune activation[3] and some measures of accelerated HIV disease progression[4 5] among highly active antiretroviral therapy (HAART)-untreated individuals. Furthermore, HIV exacerbates HSV disease in a reciprocal fashion; symptomatic reactivations and mucocutaneous shedding of HSV are greater in frequency and quantity in HIV-infected persons.[6–9]

However, it remains unclear whether the negative impacts of HSV-2 are relevant in patients with HAART-induced HIV suppression. Adverse impacts are plausible because HSV-2 could contribute to low-level HIV viremia below the limit of detection of modern assays, drive ongoing HIV replication in other anatomic compartments and/or instigate systemic inflammation through pathways independent of HIV replication. If such effects exist, then interventional trials of HSV-2 suppressive therapy in HAART-treated persons, analogous to those among HAART-naïve individuals,[10–12] would be warranted to attempt to reverse these effects. For instance, HSV-2

suppression could be a strategy for attenuating inflammation and systemic immune activation in selected patients.[13] To inform the design and interpretation of such studies, it is thus important to determine the extent to which HAART-induced HIV virological suppression might reverse HIV-related increases in HSV-2 shedding.

We, therefore, conducted a prospective cohort study of HSV (type 1 and/or 2) shedding in adults coinfected with HSV and HIV. While most literature on HSV-HIV interactions has focused on HSV-2, HSV-1 was also considered because its high prevalence, modulatory impact on the severity of subsequent HSV-2 infection,[14] similarity with HSV-2 and increasing role in genital herpes all suggest potential relevance to HIV pathogenesis.[15] Our primary objectives were to quantify HSV shedding in adults coinfected with minimal HSV symptoms, and to determine whether HAART-induced HIV suppression significantly decreases this shedding. We focused on those with infrequent herpes symptoms, because we were more interested in quantifying how much HSV shedding persists even in minimally symptomatic patients, reasoning that those with frequent herpes symptoms would already be expected to benefit clinically from anti-HSV medications. Secondary objectives were to assess the relationship between HAART and shedding of each HSV type separately, and to compare rates of HSV-1 shedding relative to HSV-2.

METHODS

Participants

We enrolled adults coinfected with HSV (type 1 and/or 2) and HIV-1 from two academic hospital-based clinics in Toronto, Ontario, Canada. Patients were eligible if they had serologically documented HIV-1 and HSV (type 1 and/or 2) infection, were aged ≥18 years, had no symptomatic oral or anogenital herpes within the previous 4 months or more than two episodes per year and were not using medications with anti-HSV activity (acyclovir, valacyclovir, famciclovir, ganciclovir, valganciclovir, cidofovir and foscarnet).

HAART participants had to have been using the same regimen for at least 90 days and have plasma HIV RNA <50 copies/mL. Plasma HIV RNA analysis was repeated at the conclusion of the 28-day study period; participants with values >1000 copies/mL were excluded.

Type-specific HSV serostatus was determined using the HerpeSelect gG-1 and gG-2 ELISA (Focus Technologies, Cypress, California, USA). The manufacturer's recommended cut-off index value of 1.1 was used to define seropositivity; sensitivity analyses using a higher cut-off were also conducted as described below.

Clinical data

Participants underwent an interview and chart review to document their clinical history, and completed brief questionnaires regarding herpes symptoms. CD4 count and HIV viral load were measured at the beginning and end of the specimen collection period. Each participant also maintained a daily log to record mild symptoms possibly related to HSV reactivations (eg, tingling or burning), since HSV symptoms often go unrecognised.[16 17]

Specimen collection

Participants were instructed on how to collect their own swab specimens from four anatomic sites (1 oral, 2 sex-specific genital and 1 anal) daily for 28 consecutive days. Oral swabs were rubbed over the upper and lower gum lines and palate. Anal swabs were inserted 1 cm into the anus and rotated for three full rotations. Men rubbed one swab the entire surface of the shaft of the penis and another swab over the urethral meatus. Women rubbed one swab over the surface of the labia majora and minora; another swab was inserted into the vagina, advanced until meeting resistance and rotated for three full rotations. Each swab was inserted into a separate vial containing viral transport media and kept refrigerated; specimens were delivered in batches to the laboratory weekly. At each weekly visit, printed, written/pictorial instructions on specimen collection and storage were provided and instructions were reinforced verbally. Study participation was terminated early in the event of a clinically confirmed herpes outbreak, with the data censored at the time of diagnosis.

HSV shedding

HSV-1 and HSV-2 shedding were detected qualitatively by PCR using the LightCycler HSV 1/2 Detection Kit (Roche Diagnostics, Mannheim, Germany). The lower limit of detection of this assay is 1 copy/µL, and any signal was reported as positive. For cost reasons, genital and anal swabs were pooled prior to testing.

Statistical considerations

Participant characteristics were summarised using descriptive statistics. HSV shedding on a given day was defined as the presence of HSV-1 or HSV-2 as detected by PCR in either the pooled anogenital or oral specimen. Type-specific shedding rates were calculated as the proportion of days on which HSV PCR was positive among seropositive participants. The median and IQR for the proportion of days with shedding was summarised according to HSV type and anatomic site.

The primary analysis was a negative binomial regression model, with the outcome equal to the number of days with any HSV shedding (type 1 or 2) for each patient, accounting for the logarithm of the number of specimen collection days using the offset function. Negative binomial models are appropriate for count data, where the conditional variances exceed the conditional means, as was the case for our data. The primary covariate of interest was HAART-induced suppression of HIV. The strategy for building multivariable models was as follows. Participant sex and baseline CD4 count were forced into the model based on prior studies suggesting them to be important

predictors of HSV shedding.[18–20] Additional variables were considered for inclusion in the multivariable model if they produced changes of ≥10% in the parameter estimate for the primary predictor, HAART. After further elimination of candidate variables due to collinearity or small cell sizes, the final multivariable model was selected based on model fit.

Secondary analyses assessed the number of days with HSV-2 shedding among HSV-2 seropositive participants, and HSV-1 shedding among HSV-1 seropositive participants using the same strategy.

The difference in the median type-specific shedding rates (HSV-2 minus HSV-1) was assessed among HSV-1 and HSV-2 dually infected participants using the Wilcoxon signed rank test, after confirming that the type-specific HSV shedding rates were similar in HSV-1 and HSV-2 dually infected and monoinfected patients.

Two sets of sensitivity analyses were conducted, because more participants than expected had no evidence of HSV shedding during the study period. First, zero-inflated negative binomial models were fit to the data; this modelling strategy can be useful for overdispersed count data, when two separate processes are postulated to contribute to observed counts (one process driving the count outcomes and a second process generating excess zeroes). Second, because false-positive HSV serology results could also have contributed to low shedding rates, analyses were also performed after increasing the threshold for defining HSV-2 seropositivity from the manufacturer-recommended index value of 1.1, to a cut-off of 3.5, as suggested in some reports.[21–23] Negative binomial models for HSV-1 shedding and HSV-2 shedding were then fit after excluding those participants with index values between 0.9 and 3.5, who also exhibited no type-specific shedding during the study period.

Finally, a third sensitivity analysis was conducted to explore the possibility that censoring participants at the time of a symptomatic herpes outbreak impacted the results, in which primary analyses were rerun after HSV shedding was considered to have occurred on all censored specimen collection days for censored HAART-naïve participants, and on no censored specimen collection days for censored HAART-treated participants.

All tests were two-sided, and a threshold of α=0.05 was used to define statistical significance. SAS V.9.3 was used for all analyses.

The target sample size was 80 participants, including 40 HAART and 40 antiretroviral-naïve individuals. Assuming an SD of the proportion of days with HSV shedding of 9.4%, based on prior data on HSV-2 shedding rates among men who are HIV positive,[1] this sample size was adequate to detect a difference in the proportion of days of HSV shedding of 6 or more with 80% power at a two-sided significance level of 0.05. For any participant who collected swabs on fewer than 14 days, an additional participant was recruited, but all participants were included in the analysis. All participants gave written informed consent prior to any study procedures.

Ethics

Ethical approval was obtained from the Research Ethics Boards of the University Health Network (approval number 08-0402-BE) and St. Michael's Hospital (09-046) in Toronto.

RESULTS

Participants

A total of 126 patients initially agreed to participate in the study, of which 41 were excluded because they could not adhere with the study protocol (n=9), could not be contacted for follow-up (n=8), withdrew consent (n=6), needed to initiate HAART (n=6), were HSV-1 and HSV-2 seronegative (n=5), had a clinical herpes reactivation (n=3), were not adherent to antiretroviral therapy (n=1), initiated chronic valacyclovir (n=1), was felt to have a local allergic reaction to the specimen collection swabs (n=1) or was enrolled in error (n=1).

No statistically significant differences were seen in sex, HIV risk factors, baseline CD4 count, baseline log10 viral load (for antiretroviral-naïve patients) and history of self-reported herpes symptoms between these 41 excluded individuals and the 85 study participants (data not shown). Comparisons for age and years since HIV diagnosis were performed separately for HAART-treated and untreated patients, since these variables may be expected to depend on HAART status, and showed that HAART-treated patients included in the study were older and had a longer duration of HIV than those not included, although the duration on HAART was similar.

Baseline characteristics of the final study cohort are shown in table 1. The antiretroviral-untreated (n=44) and HAART-treated (n=41) groups were similar, except that the HAART-treated group was older, had a longer duration of HIV infection and included relatively fewer recent immigrants to Canada. Roughly half (53%) the participants were seropositive for HSV-1 and HSV-2. All participants in the HAART group maintained HIV virological suppression during the study period as evidenced by follow-up plasma viral load measurements <50 copies/mL for 39 participants, and 71 and 77 copies/mL, respectively, for two additional participants, on the final date of specimen drop-off.

HSV shedding

In total, 8952 viral swab specimens from 2238 participant-days were collected and processed. Swabs were collected on all 28 days by 65 (77.4%) participants, on 14–27 days by 15 (17.9%) participants and on fewer than 14 days\ by 4 participants (4.8%). Reasons for having fewer than 28 swab collection days were forgetting (n=11 participants), logistical difficulties in collecting or dropping off specimens (n=2), study termination due to a clinically confirmed HSV outbreak (n=1 HAART and n=3 naïve participants), development of an unrelated serious illness

Table 1 Participant characteristics*

Variable	Antiretroviral-naïve (n=44)	HAART-treated (n=41)	p Value
Age (years)	35.4 (28.3, 43.8)	49.6 (44.7, 55.4)	<0.0001
Female n (%)	11 (25)	7 (17.1)	0.37
HSV serology			
HSV-1 pos, HSV-2 neg†	17 (38.6)	12 (29.3)	0.45
HSV-1 neg, HSV-2 pos‡	4 (9.1)	7 (17.1)	
HSV-1 pos, HSV-2 pos	23 (52.3)	22 (53.7)	
HIV risk factor§ n (%)			
MSM	28 (63.6)	29 (70.7)	0.45¶
Injection drug use	0 (0)	2 (4.9)	
MTCT	0 (0)	0 (0)	
Blood	1 (2.3)	0 (0)	
Endemic country	6 (13.6)	3 (7.3)	
Heterosexual	9 (20.5)	7 (17.1)	
Years since HIV diagnosis	2.3 (0.4, 3.9)	16.5 (10.0, 19.7)	<0.0001
Country of birth			
Canada	13 (29.6)	20 (48.8)	0.002
Other, immigrated <10 years	18 (40.9)	3 (7.3)	
Other, immigrated >10 years	13 (29.6)	18 (43.9)	
Baseline CD4 count			
Cells/mm^3	497 (388, 601)	519 (354, 691)	0.76
Percentage	27 (22, 30.8)	24 (20, 31)	0.59
Baseline HIV viral load (log copies/mL)	3.94 (3.34, 4.67)	undetectable	<0.0001
Years on HAART			
Years on current regimen	NA	2.8 (1.1, 6.7)	
Total years on HAART		8.5 (6.7, 12.2)	
HAART regimen type n (%)			
2NRTI+NNRTI	NA	8 (19.5)	
2NRTI+boosted PI		15 (36.5)	
2NRTI+unboosted PI		1 (2.4)	
3NRTI		1 (2.4)	
Other		16 (39)	
Number of days with swabs	28 (26, 28)	28 (28, 28)	0.001
History of oral herpes			
Yes	18 (40.9)	17 (41.5)	0.32¶
No	25 (56.8)	20 (48.8)	
Unsure	1 (2.3)	4 (9.8)	
History of anogenital herpes			
Yes	3 (6.8)	8 (19.5)	0.09
No	36 (81.8)	25 (60.1)	
Unsure	5 (11.4)	8 (19.5)	

*Values are median (IQR) or number (%).
†Includes two participants with equivocal HSV-2 results in the HAART group.
‡Includes one participant with equivocal HSV-1 results in the HAART group.
§Ranked according to Ontario Public Health Laboratory hierarchy.
¶Fisher's exact test.
HAART, highly active antiretroviral therapy; HSV, herpes simplex virus; MTCT, mother-to-child transmission; MSM, men who have sex with men; neg, negative; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; pos, positive.

(appendicitis, n=1) and laboratory error (n=1). More participants in the HAART group collected swabs on all 28 days (92.7%) than in the antiretroviral-naïve group (63.6%).

HSV-1 or HSV-2 shedding was observed on a total of 198 participant-days, representing 8.9% of days overall. Overall, HSV-1 shedding was observed on 4.2% of days among HSV-1 seropositive participants, while HSV-2 shedding was observed on 8.1% of days among HSV-2 seropositive participants.

HSV symptoms

Self-reported symptoms were rare, with only nine participants (10.6%) reporting any symptoms during the sampling period. Overall, symptoms were reported on only 26 of 2238 (1.2%) collection days. Site-specific shedding of HSV (type 1 or 2) coincided with site-specific symptoms on 3 of 18 collection days with oral symptoms, 5 of 7 days with genital symptoms and 0 of 1 day with anal symptoms. In none of these cases were symptoms significant enough for the participant to seek additional

investigation or treatment. Given that HSV shedding was detected on 198 specimen collection days in total, the vast majority (96%) of HSV shedding in this cohort was asymptomatic.

HSV shedding by HAART status

The proportion of participants with any HSV shedding during the study period was similar in the HAART and non-HAART groups when considering both HSV types together (58.5% vs 56.8%), HSV-1 alone (34.2% vs 29.6%) or HSV-2 alone (41.5% vs 38.6%). Crude comparisons revealed no difference in the proportion of days with shedding of any HSV, HSV-1 or HSV-2 according to antiretroviral use (table 2 and figure 1). The overall HSV shedding rate was low, with HSV type 1 or 2 detected on a median (IQR) of 3.6% (0, 14.3%) of specimen collection days, and was not significantly different between HAART users at 7.1% (0, 17.9%) and non-users at 3.6% (0, 10.7%; p=0.28). When HSV-1 and HSV-2 shedding were considered separately, shedding rates again did not differ by HAART status (table 2). The maximum shedding rate was 46.4% of collection days, observed in a female HAART-using participant who was dually infected with HSV-1 and HSV-2.

Negative binomial models were used to quantify the relationship between HAART use and shedding of either HSV type. No statistically significant relationship was observed between HAART and HSV (type 1 or 2) shedding in univariable analysis (OR=1.55, 95% CI 0.83 to 2.87) or multivariable analysis adjusted for sex, baseline CD4 count, recent immigrant status and time since HIV diagnosis (adjusted OR, aOR=1.05, 95% CI 0.43 to 2.58; table 3). Similar null results for the relationship with HAART were observed when restricting the analysis to HSV-1 shedding in HSV-1 seropositive participants, with aOR=0.70 (95% CI 0.14 to 3.47), or HSV-2 shedding in HSV-2 seropositive participants, with aOR=1.16 (95% CI 0.40 to 3.36).

Sensitivity analyses

Zero-inflated negative binomial models were fit to the data to assess for evidence of a separate process generating excess zeroes. No variables were found to be significantly associated with excess zeroes using this strategy, and qualitative conclusions of the study were unchanged (data not shown). When restricting the dataset to those participants meeting the higher threshold HSV index value definition, and building separate negative binomial models for HSV-1 shedding and for HSV-2 shedding, there was again no relationship seen between HAART and shedding of either virus type in either univariable or multivariable analysis (data not shown). When all censored specimen collection days were assumed to show HSV shedding for the three censored HAART-naive participants, but no HSV shedding for single censored HAART-treated participants, no qualitative difference in the results was observed (data not shown).

Comparison of HSV-1 and HSV-2 shedding rates

Among participants dually infected with HSV-1 and HSV-2, the median (IQR) difference in shedding rate according to HSV type (HSV-2 minus HSV-1) was 3.6% (0.0, 10.7%; p=0.009). This higher frequency of reactivations for HSV-2 is consistent with prior studies and supports greater emphasis on HSV-2 in studies of HIV coinfection. Although this comparison may be affected by the relative duration of HSV-1 and HSV-2 infection in participants who are dually infected (since both HSV types generally become less active over time), the time of infection was unknown for all study participants. The median (IQR) type-specific shedding rates were similar in participants with monoinfected HSV and dually infected HSV-1 and HSV-2 at 0 (0, 3.6%) vs 0 (0, 7.1%),

Table 2 HSV shedding rates by HAART status*

Outcome	Antiretroviral-naïve (n=44)	HAART-treated (n=41)	p Value
HSV (type 1 and/or 2) shedding rate among all participants (n=85)	3.6 (0, 10.7) range 0–33.3	7.1 (0, 17.9) range 0–46.4	0.28
HSV-1 shedding rate among HSV-1 seropositive participants (n=74)	0 (0, 3.6) range 0–33.3	0 (0, 7.1) range 0–35.7	0.38
Oral HSV-1 shedding rate	0 (0, 3.6) range 0–33.3	0 (0, 3.6) range 0–21.4	0.95
Anogenital HSV-1 shedding rate	0 (0, 0) range 0–4.8	0 (0, 0) range 0–35.7	0.06
HSV-2 shedding rate among HSV-2 seropositive participants (n=56)	3.6 (0, 10.7) range 0–33.3	7.1 (0, 14.3) range 0–46.4	0.47
Oral HSV-2 shedding rate	0 (0, 0) range 0–0	0 (0, 0) range 0–28.6	0.05
Anogenital HSV-2 shedding rate	3.6 (0, 10.7) range 0–33.3	7.1 (0, 10.7) range 0–46.4	0.55

*Values are median (IQR), range.
HAART, highly active antiretroviral therapy; HSV, herpes simplex virus.

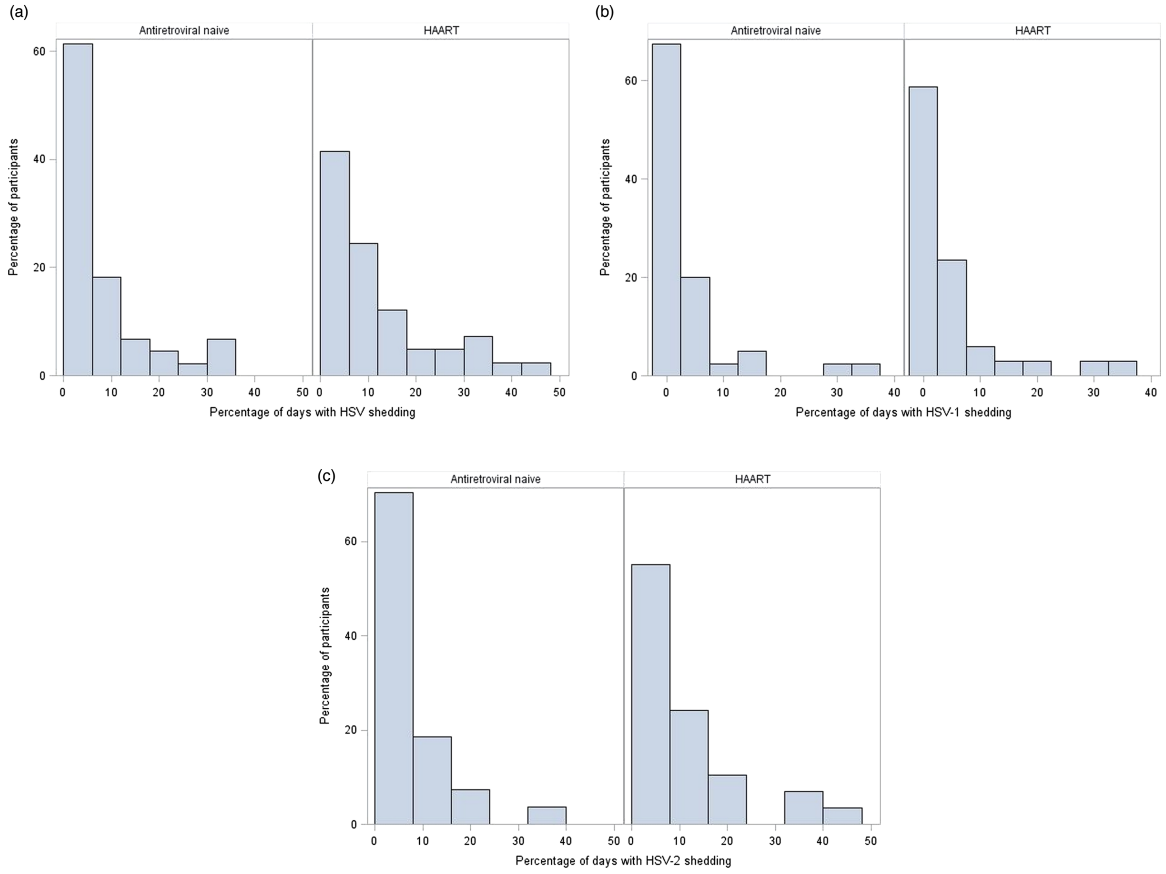

Figure 1 The proportion of participants with varying frequencies of viral shedding is shown by highly active antiretroviral therapy (HAART) status for (A) herpes simplex virus (HSV) shedding overall (both HSV-1 and HSV-2), (B) HSV-1 only and (C) HSV-2 only.

respectively for HSV-1 (p=0.29) and 7.1% (0, 10.7%) vs 3.7% (0, 11.1%) for HSV-2 (p=0.79).

DISCUSSION

This prospective cohort study documented asymptomatic HSV shedding on 7.1% of days among adults coinfected with HIV and HSV despite suppressive HAART. This value was not statistically significantly different from the shedding rate in HAART-naïve individuals. While HSV-2 reactivations were more common than HSV-1 overall, no impact of HAART on HSV shedding rates was observed for HSV-1 or HSV-2, or for both types considered together.

These findings are consistent with those of other studies, which were also unable to identify a statistically significant relationship between subclinical HSV shedding and use of antiretroviral therapy.[6 24 25] The shedding rates in this study were similar to those in a Burkinabè study, which reported HSV-2 DNA in cervicovaginal specimens at 9.7% of visits without genital ulcers among HAART-treated women and 12.7% of visits among HAART-untreated women.[25] A limitation of that study was its use of only 12 sampling times, albeit over a period of 24 weeks, compared with our strategy of sampling daily for 28 days. However, our rates were considerably lower than those in an American report, where HSV-2 shedding was observed on 17.7% (0–85.7%) of days in HAART-treated and 29.3 (0–91.5%) of HAART-untreated HIV and HSV-2 coinfected patients (p=0.08).[24] This discrepancy may be attributable to differences in participant characteristics such as baseline CD4 count, which was roughly 200 cells/mm^3 higher in our cohort. Furthermore, our study was the first to restrict the HAART-treated group to patients with undetectable HIV viral load, increasing its relevance to the modern era. Finally, our eligibility criteria required that participants have minimal herpes symptoms, because we were most interested in documenting the extent to which HSV shedding persists (and hence whether anti-HSV therapies may have a role) even in minimally symptomatic patients. As such, this cohort was likely enriched with individuals with lower rates of HSV reactivation than the broader population of individuals coinfected with HSV-2 and HIV.

That HSV shedding persists despite virological control of HIV confirms that potential adverse consequences of HSV-2 reactivations in coinfected individuals warrant further consideration. For instance, because they are characterised by lifelong latent infections with periodic reactivations, herpes viruses have been implicated as possible causes of ongoing antigenic stimulation and drivers of immune activation in HIV.[3 10 26 27] Since excess inflammation may in turn contribute to HIV disease

Table 3 Factors associated with HSV shedding rates*

Variable	Univariable model	p Value	Multivariable model	p Value
HAART				
No	1.00		1.00	
Yes	1.55 (0.83 to 2.87)	0.17	1.05 (0.43 to 2.58)	0.78
Age (per decade)	1.18 (0.90 to 1.54)	0.24		
Female n (%)				
Female	1.00		1.00	
Male	0.92 (0.43 to 1.95)	0.83	0.79 (0.36 to 1.72)	0.76
HIV risk factor—n (%)				
Non-MSM	1.00			
MSM	1.00 (0.52 to 1.94)	0.99		
Time since HIV diagnosis (per decade)	1.48 (0.97 to 2.24)	0.07	1.37 (0.74 to 2.54)	0.72
Immigrated to Canada within 10 years				
No	1.00		1.00	
Yes	0.67 (0.32 to 1.41)	0.29	0.83 (0.32 to 2.17)	0.67
Baseline CD4 count				
Cells/mm^3 (per 100)	0.99 (0.88 to 1.13)	0.96	0.99 (0.87 to 1.13)	0.71
Percentage	0.97 (0.94 to 1.01)	0.18		
Baseline HIV viral load (log copies/mL)	0.93 (0.74 to 1.18)	0.55		
History of oral herpes				
No	1.00			
Yes	1.00 (0.52 to 1.92)	0.99		
History of anogenital herpes				
No	1.00			
Yes	2.41 (1.01 to 5.77)	0.05		

*Values shown are ORs (95% CIs) for shedding of HSV-1 and/or HSV-2.
HAART, highly active antiretroviral therapy; HSV, herpes simplex virus; MSM, men who have sex with men.

progression and non-AIDS-related morbidity,[28] trials of HSV-2 suppression are warranted in coinfected, HAART-treated patients, analogous to a recent trial of cytomegalovirus suppression using valganciclovir to decrease T-cell activation.[27]

Our study has limitations that warrant consideration. First, sampling methods may not have captured all anatomic reactivations of HSV infection, and the lower limit of detection of our HSV assay was also slightly higher than that in some other reports.[24 25] Errors with specimen self-collection by study participants may also have reduced diagnostic yield. However, self-collection was used to increase study acceptability, sampling instructions were regularly reinforced using verbal and printed methods and sampling methods were the same for all participants, limiting the potential for bias. Study conclusions were unchanged in sensitivity analyses employing zero-inflated models and using more stringent criteria for the serological diagnosis of HSV infection. Second, the enrolment criterion of minimal HSV symptoms may have altered the spectrum of HAART patients included in favour of those with less HSV shedding. However, our goal was to document the extent of residual HSV shedding in the least symptomatic HAART-treated persons, and the shedding rate observed in the HAART group was numerically greater than in the naïve group. Third, since the likelihood of detecting HSV is known to increase with the duration of sample collection,[29] and because many HSV reactivations last only for a few hours,[30] our 28-daily sampling strategy may have missed rare, brief episodes of shedding. However, this issue would not be expected to impact the comparison of shedding rates, which are robust to the length of the observation windows. Fourth, the observed HSV shedding rate was lower than expected. Therefore, although the difference in shedding we observed was in the opposite direction of that expected, with more HSV shedding observed in HAART-treated than HAART-naïve participants, our sample size may have been inadequate to detect a statistically significant reduction in HSV shedding with HAART. Finally, study termination at the time of a clinical outbreak may have artificially produced lower HSV shedding rates in censored participants. However, this was uncommon and occurred in both study groups (3 naïve and 1 HAART); sensitivity analysis suggested no qualitative impact of this censoring on our conclusions.

In conclusion, we have demonstrated that reactivations of HSV-1 and HSV-2 persist in minimally symptomatic adults coinfected with HIV and are not attenuated by suppressive antiretroviral therapy. These findings support the need for clinical trials of antiherpetic therapies in HAART-treated coinfected adults, complementing those in antiretroviral-naïve populations.[10 12 31] The greater shedding rate for HSV-2 compared with HSV-1 would support a primary focus on HSV-2 in these trials, but does not exclude the potential relevance of HSV-1 as a contributing pathogen.

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