Genital symptoms and genital HSV-2 and HIV-1 shedding in co-infected women — Chiang Rai, Thailand

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Abstract

Background: There are few studies examining HIV-1 and HSV-2 shedding in HIV-1 and HSV-2 co-infected women. We examined the association between daily genital symptoms and daily HSV-2 and HIV-1 genital shedding.
Methods: Sixty-seven Thai women participating in the placebo arm of a randomized, crossover placebo-controlled trial of the effect of acyclovir on HIV-1 shedding provided daily information on genital symptoms (genital sores and other symptoms self-reported on daily diary cards). Eligible women were ages 18-49 years, had regular or no menses, had serum antibodies to HSV-2 and HIV-1, and were not eligible for antiretroviral therapy by Thai national guidelines at the time. Women self-collected genital swabs of the vaginal, vulvar and perianal region, inserted the swabs into a sponge with DNA/RNA preservative, and kept the tubes in a cooler until weekly collection. Nucleic acid extraction of both HIV-1 RNA and HSV-2 DNA were conducted and specimens with detectable virus were quantified. Associations between shedding and patient symptoms were evaluated using binomial regression with generalized estimating equations with an exchangeable correlation matrix to account for non-independence of swabs collected from the same woman.
Results: During the study, 20 (30%) participants reported burning, itching, tingling, or pain in any area on at least one day and 23 (35%) reported a sore on at least one day during the month. Of 561 swabs tested for HSV-2 DNA, 27% had detectable virus and of 525 swabs tested for HIV-1 RNA, 71% had detectable virus. Most shedding occurred in the absence of sores or symptoms (77% of swabs with detectable HSV-2 and 86% of swabs with detectable HIV-1). However, compared to swabs from women with no symptoms or sores, swabs from women with symptoms in the three-day window before collection were 2.0 (95% CI: 1.2-3.3) times as likely to be positive for HSV-2 DNA, and swabs from women with sores were 2.7 (95% CI: 1.6-4.4) times as likely to be positive. Neither symptoms nor sores reported in a three-day window prior to swab collection were associated with HIV shedding.
Conclusions: While HSV-2 shedding was more likely to occur following symptoms and sores, HIV-1 shedding was not; this difference in shedding suggests that HIV-1 shedding may be unrelated to HSV-2 mediated mucosal events. Our study found that most HSV-2 as well as HIV-1 genital shedding occurred primarily during days when there were no clinical symptoms.

Background

- HIV-1 transmission is 2-4 times more likely from persons with HSV-2 infection compared to persons without HSV-2 infection.
- HSV might increase the risk of HIV transmission due to:
  - genital vesicles and ulcers, and shedding of HIV through these breaches in epithelial integrity
  - increased HIV replication and shedding mediated by cytokine production
  - increases in co-infected HIV-infected cells to genital mucosa
- There are few studies examining HIV shedding during asymptomatic HSV-2 infection or in the presence of genital symptoms other than ulcers.
- To determine the relationship between symptoms and shedding of HSV-2 and HIV-1, we analyzed data from a study in Thailand in which women with HSV-2 and HIV-1 co-infection recorded their daily genital symptoms and collected daily vaginal swabs.

Methods

Study Population and Procedures

- 67 women co-infected with HSV-2 and HIV-1 in a randomized, placebo-controlled crossover clinical trial of suppressive acyclovir in Chiang Rai, Thailand.
  - 18-49 years with serum antibodies to HSV-2 and HIV, and not eligible for antiretroviral therapy by Thai national guidelines at the time (CD4 count < 200 cells/mL with opportunistic infection).
  - Participants randomized to a blinded study sequence of either acyclovir or placebo in the first month, a washout period in the second month (no product), and placebo or acyclovir (other product) in the third month.
- Participants submitted daily self-collected genital swabs and daily diary cards during the intervention and placebo months of the study.
  - Diary cards included questions about symptoms and behaviors (sexual intercourse, condom use, pill adherence, and exchange of ice packs).
  - Symptoms were recorded as presence of burning, itching, tingling, and/or pain (collectively) at five possible sites/events (vagina/vulva, anus/perianal area, when urinating, thighs/buttocks, and other).
  - Presence and location of sores on genitals were also recorded.
  - Participants were not provided specific teaching on HSV-2 signs and symptoms.
- Once a month, women were tested and treated for reproductive tract infections (chlamydia, gonorrhea, bacterial vaginosis, *Trichomonas vaginalis*, and *Candida albicans*) and pregnancy. A plasma specimen was collected for HIV-1 viral load at screening and study mid-point.

Swab Collection and Laboratory Methods

- Swabs were collected from the vagina, perianus and vulva each morning.
- Swabs were put into a labeled tube containing a sponge impregnated with 150 µL of AssayAssure™® (Thermo Fisher Scientific, Waltham, MA, USA) that included a DNA/RNA preservation solution.
  - Tubes were placed into a cooler with frozen ice packs changed twice daily and temperature monitoring strips (3M Monitor Mark, Cold Chain Technologies, Holliston, MA, USA) and brought to weekly study visits. Swabs were then stored at -70ºC and shipped frozen to the laboratory for evaluation
- Genital swab specimens were thawed and the sponge and swab were squeezed to release all fluid. Next, 600 µL of Amplicor Monitor kit lysis buffer was added to 150 µL of the fluid from each swab; this 750 µL of eluate was used for nucleic acid extraction of both HIV-1 RNA and HSV-2 DNA.
- Nucleic acid extraction procedures were modified by adding freshly prepared dithiothreitol (DTT) to 750 µL of sample eluate to obtain a final concentration of 0.3 mM. The sample was heated at 60 ºC for 10 minutes. The remaining extraction steps followed the manufacturer's instructions. Specimens with detectable HIV and HSV-2 were also evaluated for quantity of virus reported as copies/swab. HIV RNA was quantified using Amplicor HIV-1 Monitor version 1.5 (Roche Molecular Systems, Inc., Branchburg, NJ, USA), with a lower limit of detection of 40 copies/swab and lower limit of quantification of 80 copies/swab. HSV-2 DNA was quantified using an in-house TaqMan-based real-time duplex polymerase chain reaction (PCR) assay described previously.The lower limit of detection was 40 copies/swab and the lower limit of quantification was 400 copies/swab.
- We analyzed viral shedding results from a subset of swabs which included those collected on study visit days 7, 14, and 21, plus a random selection of 1-2 additional swabs per week during the placebo month.

Statistical Analysis

- Shedding from swabs defined as a binary outcome (detectable or non-detectable). The exposures of interest were symptoms (burning itching, tingling, and/or pain at a genital site) and/or sores. The exposure variable was categorized as no symptoms or sores, symptoms only, sores only, and symptoms and sores in order to assess the associations between symptoms and shedding, and sores and shedding.
  - Three different time intervals were used to define the presence or absence of symptoms and sores: the day of swab collection, a three-day window (the day of swab collection plus the two preceding days), and a seven-day window (the day of swab collection plus the six days preceding days).
- Associations between shedding and patient characteristics were evaluated using binomial regression with generalized estimating equations with an exchangeable correlation matrix to account for non-independence of swabs collected from the same woman.
- Separate binomial regression models were used to evaluate the associations between symptoms and sores and dichotomous variables for 1) HSV-2 shedding and 2) HIV shedding. Effect modification was assessed first by creating an interaction term between the exposure and potential modifier. If the interaction term had a Wald P value < .05, it was retained in the model. Variables were considered potential confounders if they were related to both the exposure and the outcome and were not effect modifiers. The model was reduced using a backwards elimination strategy. Starting with the variable with the highest Wald P value, variables were removed from the model if the estimated prevalence ratio between symptoms/sores and shedding changed by less than 10% from the unadjusted association.12The quantity of virus shed was also analyzed using binomial regression models.
- All analyses were done using SAS 9.3 (SAS Institute Inc., Cary, NC, USA.)

Results

- Median age of participants was 33 years (Table 1).
- Median time since HIV diagnosis was 5 years, median CD4 count was 366 cells/uL, and median plasma viral load was 4.6 log copies/mL.
- Before enrollment, 17 (25%) participants knew they had genital herpes, mostly due to symptoms or notification by their healthcare provider.
  - Among these, the median time since they knew they had genital herpes was 3 years.
- Most participants (60%) reported ever having symptoms consistent with genital herpes before enrollment.
  - The most common symptoms were genital itching or tingling (95%) and genital sores (85%).
  - Most symptoms were vaginal (90%), but 20% reported symptoms at more than one site.
- 8 (12%) tested positive for bacterial vaginosis, 2 (3%) for *T. vaginalis*, and 14 (21%) for *C. albicans*.

Table 1. Baseline characteristics of 67 HIV/HSV-2 co-infected women, Chiang Rai, Thailand

Characteristic	Median (range) or n (%)[a]
Age (years)	33 (22–46)
Years since HIV diagnosis	5 (1–15)
CD4 count (cells/µL)	366 (209–930)
HIV-1 plasma viral load (log copies/mL)	4.6 (2.9–5.8)
Knew they had genital herpes before enrollment	17 (25%)
Knew they had genital herpes based on (n=17)	
Symptoms	13 (77%)
Told by a provider	8 (47%)
Partner has herpes	2 (12%)
Other	4 (24%)
Time since they knew they had genital herpes (n=17)	3 years (4 months–10 years)
Ever had symptoms consistent with genital herpes	40 (60%)
Time since last symptom (n=40)	3 months (1–4 months)
Symptom (n=40)	
Genital itching or tingling	38 (95%)
Genital sore	34 (85%)
Genital pain	23 (58%)
Genital burning	23 (58%)
More than one symptom	35 (88%)
Site of symptom (n=40)	
Vagina	36 (90%)
Buttocks	7 (18%)
Anal/perianal	3 (8%)
Upper thighs	3 (8%)
More than one site	8 (20%)

Table 3. Adjusted[a] prevalence ratios between symptoms and/or sores[b] and HSV-2 and HIV-1 shedding on self-collected genital swabs, Chiang Rai, Thailand

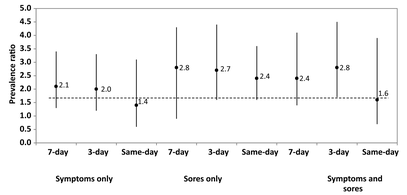
Symptoms and/or sores	HSV-2 shedding[c] (N=561) aPR (95% CI)	HIV shedding[c] (N=525) aPR (95% CI)
No symptoms, no sores	1.0 (ref)	1.0 (ref)
Symptoms only	2.0 (1.2-3.3)[d]	1.4 (0.6-3.2)
Sores only	2.7 (1.6-4.4)[d]	1.1 (0.4-2.8)
Sores and symptoms	2.8 (1.7-4.5)[d]	0.7 (0.3-2.2)

Abbreviations: aPR, adjusted prevalence ratio; CI, confidence interval; ref, referent
[a] Adjusted for HIV plasma viral load
[b] Defined as presence of prodromal symptoms and/or sores on diary cards in the two days prior to and day of swabs collection (three-day window before swabs collection).
Symptoms were defined as burning, itching, tingling, or pain at any site/event (vagina/vulva, anus/perianal area, when urinating, thighs/buttocks, or other); sore was defined as sore at any site (vagina/vulva, anus, buttocks, thighs, or other). Symptoms and/or sores categories are mutually exclusive.
[c] Defined as binary outcome, with non-detectable viral shedding considered no shedding and a non-quantifiable or quantifiable viral shedding considered shedding.
[d] P < .05

Table 2. Characteristics associated with HSV-2 and HIV-1 shedding[a] on genital swabs self-collected from 67 women during the placebo month, Chiang Rai, Thailand.

	HSV-2 shedding (N=561)		HIV-1 shedding (N=525)	
Characteristic	n (%)	PR (95% CI)	n (%)	PR (95% CI)
Overall	149 (26.6)		370 (70.5)	
Baseline characteristics				
Age group (years)				
22-30	19 (18.8)	1.0 (ref)	66 (68.0)	1.0 (ref)
30-39	94 (28.1)	1.5 (0.7–3.3)	220 (69.6)	1.0 (0.8–1.3)
40-46	36 (28.8)	1.5 (0.6–3.6)	84 (75.0)	1.1 (0.8–1.5)
Baseline HIV-1 plasma viral load (log_{10} copies/mL)				
≤ 4.0	16 (11.0)	1.0 (ref)	42 (30.7)	1.0 (ref)
4.0-5.0	88 (31.7)	2.5 (1.2–5.0)[c]	206 (78.9)	2.3 (1.6–3.5)[c]
> 5.0	45 (32.9)	2.3 (1.0–5.2)[c]	122 (96.1)	2.9 (1.9–4.2)[c]
Knew they had genital herpes before enrollment				
No	113 (27.2)	1.0 (ref)	273 (69.8)	1.0 (ref)
Yes	36 (24.7)	0.9 (0.5–1.6)	97 (72.4)	1.0 (0.8–1.3)
Ever had symptoms consistent with genital herpes				
No	62 (27.1)	1.0 (ref)	161 (73.5)	1.0 (ref)
Yes	87 (26.2)	1.0 (0.6–1.7)	209 (68.3)	1.0 (0.8–1.2)
Placebo month characteristics				
CD4 count (cells/µL)[d]				
209-350	71 (25.0)	1.0 (ref)	216 (81.8)	1.0 (ref)
351-500	65 (38.7)	1.5 (0.9–2.6)	107 (67.7)	0.8 (0.7–1.0)[c]
501-930	13 (11.9)	0.5 (0.3–1.0)	47 (45.6)	0.6 (0.4–0.9)[c]
Phase of menstrual cycle[e]				
Follicular	29 (27.6)	1.0 (ref)	81 (77.1)	1.0 (ref)
Periovulatory	39 (22.9)	0.8 (0.5–1.2)	118 (69.4)	0.9 (0.8–1.0)
Luteal	51 (24.6)	0.8 (0.5–1.3)	144 (69.6)	0.9 (0.8–1.0)
HIV shedding[a]				
No	25 (16.1)	1.0 (ref)	--	--
Yes	114 (30.8)	1.4 (1.0–1.9)		
Symptoms and/or sores[b]				
No symptoms or sores	114 (22.9)	1.0 (ref)	320 (69.0)	1.0 (ref)
Symptoms only	10 (43.5)	2.0 (1.2–3.3)[c]	21 (91.3)	1.3 (0.8–2.3)
Sores only	15 (65.2)	2.7 (1.6–4.4)[c]	17 (77.3)	1.1 (0.6–2.1)
Symptoms and sores	10 (62.5)	2.8 (1.7–4.5)[c]	12 (75.0)	0.8 (0.3–1.8)

Figure 1. Prevalence ratios and 95% confidence intervals for HSV-2 shedding with 7-day, 3-day, and same-day windows for symptoms/sores prior to swab collection



Conclusions

- HSV-2 shedding was more likely when women reported symptoms in the three-day window preceding swab collection compared to when they had no preceding symptoms.
- Most HSV-2 shedding occurred on days when there were no associated symptoms or sores.
- Neither symptoms nor sores were associated with presence of HIV shedding.