Cyclic Changes in HIV Shedding From the Female Genital Tract During the Menstrual Cycle

Marcel E. Curlin,¹,² Wanna Leelawiwat,³ Eileen F. Dunne,⁴ Wannee Chonwattana,² Philip A. Mock,² Famui Mueanpai,² Sukhon Thep-Amnuay,⁴ Sara J. Whitehead,¹,² and Janet M. McNicholl¹

Factors increasing genital human immunodeficiency virus (HIV) shedding may increase female-to-male HIV transmission risk. We examined HIV shedding in 67 women with HIV type 1 and herpes simplex virus type 2 coinfection, during 2 menstrual cycles. Shedding occurred in 60%, 48%, and 54% of samples during the follicular, periovulatory, and luteal phases, respectively (P = .01). Shedding declined after menses until ovulation, with a slope \(-0.054 \log_{10} \text{copies/swab/day} (P < .001)\), corresponding to a change of approximately 0.74 \(\log_{10}\) copies between peak and nadir levels. Shedding increased during the luteal phase only among women with CD4 counts of <350 cells/\(\mu\)L. In reproductive-aged women, shedding frequency and magnitude are greatest immediately following menses and lowest at ovulation.

**Keywords.** HIV; mucosal; shedding; female; genital tract; menstrual cycle; transmission; Thailand.

Worldwide, most new cases of human immunodeficiency virus (HIV) infection among adults occur during heterosexual exposure, while maternal-to-child transmission is the primary cause of new infections among children. Transmucosal infection is therefore a critical driver of the global HIV epidemic. The risk of mucosal transmission is correlated with both plasma and genital HIV RNA levels, and each \(\log_{10}\) increase in genital HIV type 1 (HIV-1) RNA is associated with a 2.20-fold increase in the risk of female-to-male transmission [1]. It is therefore of considerable importance to HIV prevention efforts to elucidate biological variables and exogenous factors that influence HIV-1 shedding from the female genital tract.

In reproductive-aged women, one potentially important factor affecting viral shedding is the physiologic fluctuation in hormone levels and associated changes occurring during the menstrual cycle. Recent studies suggest that women using hormonal contraceptives may be more likely to acquire and transmit HIV than women not using hormonal contraceptives. Although these findings may be due partly to behavioral differences, these and earlier results draw attention to the possibility that circulating hormone levels may affect viral shedding from the genital tract. However, human studies directly examining the relationship between menstrual cycle phase and genital shedding have been inconsistent, with some reports failing to demonstrate any correlation between menses and shedding [2–4] and others indicating a significant cyclic relationship, typically with peak shedding levels immediately prior to or during menses and nadir levels around the time of ovulation [5–7].

Interpretation of the literature in this area is challenging because of methodological differences between studies, and the large number of biological and behavioral variables that may influence actual or apparent shedding levels. Current studies typically rely on amplification of HIV nucleic acids as a surrogate measure of shedding of infectious virus particles. However, the frequency and quantity of shedding vary by sampling site (ie, vagina, posterior fornix, ectocervical mucosa, and endocervical canal) [3, 6, 8], by sampling method (eg, swabs, wicking strips, tampons, lavage, direct aspiration of fluid, and cytobrush) [5, 8], by amplification of RNA versus DNA, and by downstream processing factors. Biologically, genital tract shedding is significantly but imperfectly correlated with plasma RNA levels [3, 4, 9] and may be upregulated by pregnancy, selenium deficiency, herpes simplex virus type 2 (HSV-2) reactivation, and alterations of vaginal flora. Shedding is decreased by antiretroviral therapy use, and recovery of nucleic acids may be reduced by douching [6].

Thus, biological factors, heterogeneous study populations, lack of consensus on methodological approaches, and, in many instances, limited sampling [2–4, 6, 7] have resulted in discordant outcomes, and consequently no single study has provided a definitive characterization of the relationship between stage of menstrual cycle and genital shedding. We previously examined the impact of acyclovir therapy on genital tract HIV-1 shedding in 67 Thai women coinfected with HIV-1 and HSV-2 [10, 11]. Here, we examine the effect...
of menstrual cycle on HIV-1 shedding from the genital tract in these women.

MATERIALS AND METHODS

Ethics Approval
This study was approved by the institutional review board of the US Centers for Disease Control and Prevention and by the Ethics Review Committee of the Thai Ministry of Public Health (clinical trials registration NCT00362596).

Study Population
Sixty-seven HIV-1/HSV-2–coinfected women enrolled in a triple-blind, placebo-controlled, randomized crossover trial to evaluate the effect of acyclovir on HIV shedding. Enrollment criteria included female sex, age 18–49 years, regular or no menses, serum antibodies to HIV-1 and HSV-2, and ineligibility for ART according to local guidelines [10]. Pregnant women and women with genitourinary tract infections were excluded. Women enrolled in the study at the start of menses. At enrollment, participants were randomized to receive either acyclovir 800 mg/day or placebo orally for 1 month, followed by a washout period of 1 month and receipt of the alternate therapy for 1 month.

Quantification of Genital Shedding
Participants performed self-collected vaginal swabs (SCS) each morning during the first and third months of the study. For each cycle, SCS collection began on the day after menstrual bleeding stopped and continued until onset of next menses. Women were instructed to collect SCS by inserting a sterile Dacron swab approximately 2 inches into the vaginal canal and then swabbing the vulvar, perianal, and rectal areas. Swabs were placed in 150 μL of DNA/RNA storage medium (AssayAssure, Sierra Molecular, Sonora, CA) and stored at temperatures near 20°C in coolers containing ice packs and temperature monitoring strips (range, 8°C–31°C). Coolers were brought to the clinic at the next scheduled weekly visit, where temperatures were recorded, and samples were stored at −70°C until testing. To characterize viral shedding over time, we selected samples collected 1–3 days apart to evenly cover the sampling period in each woman. Swab HIV-1 RNA content was measured using the Amplicor HIV-1 Monitor kit, version 1.5 (lower limit of detection, 40 copies/swab; lower limit of quantification, 80 copies/swab), as described elsewhere [11]. For the present analyses, we excluded samples exposed to temperatures exceeding 31°C at any time before testing, samples for which amplification of the kit internal control failed, samples from amenorrheic women, and samples with visible blood contamination.

Definition of Luteal and Follicular Phases
The onset of menstruation was self-reported by study participants. Ovulation (“day 0”) and the beginning of the luteal phase was assumed to occur 14 days before onset of menstruation, and the follicular phase accounted for the residual length variation of the menstrual cycle [12]. For shedding frequency calculations, the menstrual cycle was divided into a periovulatory phase (estimated day of ovulation ±3 days), a luteal phase (end of periovulatory phase to onset of menses), and a follicular phase (end of menses to beginning of periovulatory phase).

Statistical Analysis
Detectable viral loads below the level of quantification were set at 40 copies/swab, while undetectable values were set at 0 copies/swab. To assess the sensitivity of the analyses on the left-censored data, we also assigned random values between 41–80 and 0–40 copies/swab to nonquantifiable and undetectable values, respectively. We used the nonparametric LOWESS smoother function [13] to evaluate the relationship between mucosal HIV RNA levels and cycle phase. To test the hypothesis of a decline in HIV-1 shedding during the follicular phase and an increase in HIV-1 shedding during the luteal phase, we computed a piecewise linear spline for HIV-1 RNA level, with a single knot at cycle day 0. Adding the spline to the linear term in the regression model provides a statistical test of the change in slope for days >0 versus the slope for days ≤0. A mixed linear regression model was used for analyses incorporating random intercept and slope. For each participant, the dependence structure for sampling visits was assumed to be exchangeable (ie, we assumed a common correlation between repeated observations). Statistical inferences were based on robust standard errors to account for possible model misspecifications. Detectable HSV-2, hormonal contraceptive use, and acyclovir treatment were included in the model as covariates. The models were computed using SAS statistical software (PROC MIXED, SAS 9.1, SAS, Cary, NC) and R (available at: http://www.r-project.org/).

RESULTS

Study Population and Sample Collection
Among 67 women with specimens analyzed (Table 1), the median baseline CD4 T-lymphocyte count at screening was 366 cells/μL (range, 209–930 cells/μL). The median plasma viral load was 4.6 log_{10} copies/mL (range, 2.9–5.84 log_{10} copies/mL). During the study, 18 women (28%) reported hormonal contraceptive use, including oral contraceptives (n = 12), injectable progesterone-based contraception (n = 5), or both (n = 1). Five women reported having no menses (4 were receiving hormonal contraceptives). The median menstrual cycle length was 28 days (range, 23–29 days) while receiving placebo and 29 days (range, 22–41 days) while
We evaluated the pattern of HIV-1 RNA shedding across the menstrual cycle in 67 HIV-1/HSV-2–coinfected women. We found a significant cyclic variation in mucosal HIV-1 shedding, with peak shedding frequency and intensity during the follicular phase and nadir levels around the time of ovulation. During the luteal phase, shedding rose significantly only in women with CD4 counts of <350 cells/µL. Acyclovir did not affect shedding patterns but was associated with a significant decrease in HIV-1 shedding levels and frequency. Shedding variation across the cycle was not affected by the use of hormone-based contraceptives.

To our knowledge, our study is the largest to examine viral shedding across the menstrual cycle. Our results concur with those of 3 earlier studies noting periodicity in genital shedding of HIV-1 in relation to the menstrual cycle [5–7]. However, at least 2 other studies have failed to note any clear relationship: Mostad et al found no correlation between menstrual shedding of HIV-1 DNA among 17 women undergoing daily vaginal and cervical swabbing over 4 weeks [3], and Villanueva et al similarly found no correlation in their analysis of RNA shedding data from 25 women undergoing weekly cervicovaginal lavage over 4–8 weeks [2]. These negative results were obtained despite confirmation of cycle stage by use of hormone level measurements. Differences in study population, cohort size, sampling frequency, and detection methods may be contributing factors.

There are important limitations to this study. Because of the original study design, appropriate samples were not available to measure circulating hormone levels to confirm ovulation and stage of menstrual cycle, and it is reasonable to assume that some cycles were anovulatory [7, 14]. Past studies have reported that the inclusion of women with anovulatory cycles has obscured detection of a significant relationship between cycle stage and plasma HIV RNA levels [15]. At least one prior study has noted a correlation between cycle phase and cervical but not vaginal shedding [6]. In this study, sampling was performed using vaginal swabs, and we therefore had no opportunity to examine the contribution of the cervix or other sites in isolation, although they may have contributed to the shedding observed. These limitations might be expected to hinder detection of a relationship between cycle stage and shedding in our cohort, and it is therefore noteworthy that we have nevertheless observed significant cyclic shedding in our participants. Similar limitations are likely to be present in HIV prevention study cohorts, where biochemical verification of ovulation and semiinvasive genital sampling are not routine.

We found that shedding declined during the follicular phase by 0.054 log10 per day. Assuming a 14-day follicular phase, this corresponds to a 0.75 log difference between peak and nadir shedding levels. The significance of this difference would be expected to hinder detection of a relationship between cycle phase and shedding in our cohort, and it is therefore noteworthy that we have nevertheless observed significant cyclic shedding in our participants. Similar limitations are likely to be present in HIV prevention study cohorts, where biochemical verification of ovulation and semiinvasive genital sampling are not routine.

We found that shedding declined during the follicular phase by 0.054 log10 per day. Assuming a 14-day follicular phase, this corresponds to a 0.75 log difference between peak and nadir shedding levels. The significance of this difference
for transmission risk is not clear, although one recent estimate suggests that it would be associated with a 1.65-fold difference in the risk of female-to-male transmission of HIV [1]. The cyclic nature of HIV shedding in the female genital tract must be considered in studies examining HIV transmission and correlates of the risk of HIV transmission during heterosexual contact.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank the study participants, who made this research possible.

Financial support. This work was supported by the US Centers for Disease Control and Prevention.

Potential conflicts of interest. All authors: No reported conflicts.
All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


