Frequent Genital HSV-2 Shedding Among Women During Labor in Soweto, South Africa

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Abstract

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in Soweto, South Africa

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Background: Despite high herpes simplex virus type 2 (HSV-2) incidence and prevalence in Africa, we are unaware of published neonatal herpes reports. To assess the potential for neonatal transmission we investigated the frequency of established risk factors including HSV acquisition in late pregnancy and HSV shedding during labor in South Africa.

Methods: Women in labor at a Soweto hospital underwent HSV serologic testing and genital swab collection for HSV PCR. Women initially HSV-2 seronegative were assessed for seroconversion 4-6 weeks post-partum.

Results: Of 390 women enrolled, 58.7% were HSV-2 seropositive. Genital HSV was detected in 22.6% of HIV-coinfected vs. 11.8% of HSV-2-positive/HIV-negative women (RR,1.91; 95% CI,1.04-3.53; *P*=0.038). We identified no women who recently acquired HSV-2.

Conclusion: HSV-2 reactivation occurs frequently among South African women during labor. Lack of reported neonatal herpes despite exposure during delivery suggests the possibility of virologic or immunologic differences that decrease the risk of neonatal infection.

Introduction

Neonatal herpes is one of the most deadly infections in the newborn. Untreated, the mortality is 50% when the central nervous system (CNS) is involved and 85% for disseminated infection, with up to 50% of survivors demonstrating developmental abnormalities at one year.[1,2] Administration of high-dose acyclovir decreases the mortality rate to 4% for CNS disease and 29% for disseminated disease.[2] Diagnosis of neonatal herpes, however, is challenging: vesicles are absent in nearly 40% of severe infections,[2,4] other early symptoms and signs are non-specific, and disseminated infection can appear identically to bacterial sepsis.[3]

In the U.S., incidence estimates of neonatal herpes range from 1 case per 1,700 live births to 1 case per 12,500 live births.[3] This risk is more than 300 times higher when herpes simplex virus (HSV) is isolated vs. not isolated from the genital tract during labor.[5] More than half of neonatal herpes cases in the U.S. and Europe are associated with maternal acquisition of HSV-1 or HSV-2 near the time of delivery and the remainder result from exposure of the baby to reactivating maternal infection.[5-7] The rate of transmission to the infant is higher (25-50%) in first episode infections than reactivation (<1%), presumably due to the lack of transplacentally transferred maternal neutralizing antibodies and increased quantity of HSV shed in maternal first episode infections.[3,5,7,8]

In South Africa, HSV-2 seroprevalence among women of reproductive age is up to 70%, more than twice as high as the U.S., where the seroprevalence is 21%.[9-12] If more young women are already infected with HSV-2 prior to pregnancy, the risk of neonatal herpes in South Africa could be lower since fewer infants would be exposed to first episode infection at birth. HSV-2 incidence in southern Africa remains high throughout the reproductive years, however, at 8.8 cases/100 person-years among women 18-24 years of age and 5.3 cases/100 person-years among women 35 years or older.[13] This is higher than the U.S. where estimated HSV-2 incidence is 2.25/100 person-years among women 20-29 years of age and 1.73/100 person-years among women age 30-39.[14] As 1.0% of HSV-2 seronegative women in the U.S. seroconvert during pregnancy,[7] this suggests that South African women may also be at risk of HSV-2 infection during pregnancy. There are few published reports of neonatal herpes cases from the developing world, however, and to our knowledge, no reports have been published from Africa. This may reflect a lower incidence of neonatal herpes in developing countries or underdiagnosis and

underreporting. The nonspecific symptoms and signs of neonatal herpes, coupled with the expense of laboratory testing for HSV in environments with a high burden of competing infectious diseases, suggests the latter as a possibility. We performed a prospective cohort study to determine the frequency of HSV-2 acquisition among South African women in late pregnancy and the frequency of HSV shedding from the genital tract of women during labor. The objective was to assess the potential for neonatal HSV transmission among South African women.

Materials and Methods

Study Population

We recruited women in early labor at the Chris Hani Baragwanath Maternity Hospital in Soweto, South Africa, including women undergoing induction. Entry criteria included women ≥18 years of age who were expected to have a vaginal delivery, known HIV status or plan for testing prior to delivery, ability to return to clinic in 4-6 weeks, ability to read and understand the consent form in English, Zulu, or Sotho, and willingness and ability to give written consent. The protocol was approved by the Institutional Review Boards of the University of the Witwatersrand and the University of Washington and all participants signed an informed consent.

Clinical and Laboratory Procedures

Baseline clinical data including prenatal rapid plasma reagin (RPR) result, HIV status, and for HIV-positive women, the most recent CD4 count and current antiretroviral drugs, were extracted from the participant's medical record. Participants were interviewed regarding history of genital ulcer disease and to confirm their antiretroviral regimen.

A Dacron swab was used to sample the genital skin and mucosa of the vagina, vulva, and perineum.[15,16] Participants were examined for genital lesions and a separate swab was used to sample any lesions identified. Swabs were placed in a vial containing 1 mL of PCR digestion buffer and stored at room temperature. At the University of Washington, DNA was extracted from 200 µL of buffer with a QIAamp 96 DNA Blood Kit (Qiagen) following the manufacturer's recommendations.[17] Quantitative real-time PCR was performed (QuantiTect multiplex PCR master mix from Qiagen) with a 7900HT sequencing detection system using a validated assay with common primers to the HSVgB region.[17,18] A positive

assay was defined as detection of ≥150 copies of HSV DNA/mL of swab fluid.[18] Positive samples were analyzed with type-specific primers to distinguish HSV-1 from HSV-2.[19]

Blood was drawn for HSV-2 serology. The Kalon HSV-2 gG2 ELISA assay was performed in South Africa. Results were reported according to the manufacturer's specifications for index value cut-offs: >1.1 was positive, <0.9 was negative, and 0.9-1.1 was reported as equivocal. The serum was stored at -20° C and shipped to the University of Washington for confirmatory HSV Western blot testing.[20]

Delivery data were extracted from participants' charts. At 10-14 days post-partum, participants were contacted by phone to assess the clinical status of the neonate. At the 4-6 week post-partum visit, the mother was interviewed about the interim medical history of the infant, including any medical evaluations for illness since birth, and the infant was examined. Women with negative or equivocal HSV-2 results by the Kalon assay at enrollment underwent repeat HSV Western blot to evaluate for seroconversion. If a participant did not return for the scheduled post-partum visit, attempts were made to contact the participant by phone; if unable to reach the participant, in those who had agreed to home visits, staff would drive to the participant's home.

Definitions

HSV serostatus was defined by HSV-1 and HSV-2 antibody profiles obtained by Western blot.[20] Participants were classified into the following categories: HSV seronegative, HSV-1 seropositive only, HSV-2 seropositive only, and both HSV-1 and HSV-2 seropositive. HSV-2 seropositive women, therefore, included women only HSV-2 seropositive and women who were both HSV-1 and HSV-2 seropositive. Seroconversion was defined as a change in HSV status between acute (delivery) and convalescent (4-6 weeks post-partum) sera. For participants with atypical Western blot profiles,[21] acute and convalescent profiles were compared to assess for progressive acquisition of HSV bands. If no change was demonstrated, the results were considered negative. Most women found to seroconvert will have acquired HSV in the last trimester of pregnancy as 68% of persons with pre-existing HSV-1 antibodies who acquire HSV-2 seroconvert within 3 months.[21] The follow-up time was selected to capture participants infected with HSV-2 in late pregnancy rather than in the post-partum period.[21]

Statistical Analysis

The primary endpoint was the frequency of HSV acquisition in late pregnancy as determined by HSV shedding from the genital skin and mucosa of women lacking antibodies to the same HSV type, or seroconversion. Secondary endpoints were the frequency of genital HSV shedding in women with antibodies to the same HSV type and, among HSV-2 seropositive women, the risk of HSV-2 shedding for HIV-positive compared with HIV-negative women. Confidence intervals for proportions were obtained by the Agresti-Coull method.[22] When no events were observed, confidence intervals were determined by the mid-P method.[23] Poisson regression with robust variance estimates was used to determine the relative risk of genital HSV shedding for HIV-positive compared with HIV-negative women among those HSV-2 seropositive, and the relative risk of HSV-2 seropositivity for HIV-positive compared with HIV-negative women. The following covariates were tested for univariate association with genital HSV shedding and HSV-2 seropositivity: age, prior pregnancy, CD4 count, highly active antiretroviral therapy (HAART: defined as ≥ three antiretroviral drugs), and HAART regimen containing tenofovir, an antiretroviral that inhibits HSV DNA polymerase.[24] Variables significant at $P < 0.2$ were included in a multivariate model. Backwards elimination was used to remove covariates not significantly ($P < 0.05$) associated with each outcome. The mean quantity of HSV DNA for positive samples was compared for HIV-positive and HIV-negative women by t-test.

Among HSV-2 seropositive women, Poisson regression was also used to determine the relative proportion of women reporting a history of genital ulcer disease for HIV-positive compared with HIV-negative women. The same covariates were tested for univariate association with a history of genital ulcer disease and variables significant at $P < 0.2$ were included in a multivariate model. Backwards elimination was used to remove covariates not significantly ($P < 0.05$) associated with the outcome. Among HSV-2 seropositive women reporting a history of genital ulcer disease, the mean number of episodes in the past year was compared for HIV-positive and HIV-negative women by t-test allowing for unequal variances. Among HSV-2 seropositive women, the relative risk of having genital lesions during labor for HIV-positive compared with HIV-negative women was also estimated by Poisson regression; this analysis could not be adjusted due to the small number of participants with genital lesions. Analyses were performed using Stata 12.0 (StataCorp, College Station, TX).

Calculations of study precision estimated that between 239 to 474 participants would be required to estimate the proportion of women with recent HSV acquisition (in the past 3 months) with 1% precision, assuming a HSV-2 seroprevalence of 50%[9-11,25] and an annual HSV-2 incidence between five to ten cases/100 person-years.[13]

To investigate whether HSV-2 seronegative women who did not return for repeat HSV serology may have been more likely to seroconvert than those who did, we assessed whether there were differences between groups in measured risk factors by performing t-tests for continuous variables (age, gravidity, and parity) and a chi-square test for HIV-status.

Results

We enrolled 390 women from whom we collected serum for HSV serology and genital swabs for HSV PCR. The median age of participants was 26 (range, 18-44) years; 135 (35.4%) were primigravid (Table 1). Of the 387 women with known HIV status, 132 (34.1%) were HIV-positive, with a median CD4 count (obtained a median of 3 months prior to enrollment) of 321 (range, 18-1237) cells/μL. All but one were receiving antiretroviral therapy, including 52 (39.7%) who were receiving HAART and 78 (59.5%) who were receiving antiretroviral drugs to prevent mother-to-child transmission. Five (1.3%) of 377 women had a positive RPR during the prenatal period, only one of whom reported a history of genital ulcers; this participant was also HSV-2 seropositive. No women were taking acyclovir as HSV suppressive therapy near term is not the standard of care for women with genital herpes in South Africa.

Baseline HSV-2 serostatus and history of genital ulcer disease

HSV-2 seropositivity, as determined by Western blot, was present in 229 women (58.7%), including 116 (87.9%) of 132 HIV-positive and 111 (43.5%) of 255 HIV-negative women (Table 2). HSV-2 seroprevalence increased with age (Figure). Of the 29 women who reported a history of genital ulcers, 22 (76%) were HSV-2 seropositive and reported a mean of four (range, 0-24) episodes in the past year compared to a mean of one episode (range, 0-2) for HSV-2 seronegative women. A history of genital ulcer disease was more common among HSV-2 seropositive women co-infected with HIV compared with HIV-negative women (14.2% vs. 5.5%; $P = 0.040$; Table 3). Among HSV-2 seropositive women with a history of genital ulcer disease, the mean number of episodes in the past year for HIV-positive compared with HIV-negative women was not significantly different (13 of 16 HIV-positive/HSV-2 seropositive women

with a history of genital ulcer disease reported a mean of two episodes compared to nine episodes for six HIV-negative/HSV-2 seropositive women).

Genital HSV shedding

Genital HSV shedding was detected in none of 161 HSV-2 seronegative women (95% CI, 0-1.8%). Among HSV-2 seropositive women, genital HSV was detected in 17.2% (39 of 227) and was typed as HSV-2 in all cases; 95% (37 of 39) of shedding episodes were subclinical. The risk of HSV shedding among HSV-2 seropositive women was higher among HIV-positive compared with HIV-negative women (22.6% vs. 11.8%; relative risk, 1.91; 95% CI, 1.04-3.53; $P = 0.038$). The mean quantity of HSV-2 DNA was similar among HIV-positive and HIV-negative women (4.57 vs. 4.42 $\log_{10}$copies/mL; $P = 0.80$). Lesions were identified in three (2.6%) of 116 HSV-2/HIV-coinfected women compared with four (3.6%) of 111 HSV-2 seropositive/HIV-negative women. HSV DNA was detected from only two women with lesions. Genital swabs were collected a median of one day prior to delivery (range, 0-50 days). Subgroup analysis, including only those women for whom swabs were collected within one day of delivery, provided similar results: among HSV-2 seropositive women, HSV shedding was detected in 23% of 61 HIV-positive compared with 10% of 58 HIV-negative women.

HSV-2 seroconversion

Post-partum serology was available for 91 (56.5%) of 161 HSV-2 seronegative women, collected at a median of 42 (range, 25-78) days after delivery. None seroconverted (95% CI, 0-3.2%). We found no significant differences in age, gravidity, parity, or HIV-status at baseline between HSV-2 seronegative participants who did and did not undergo post-partum HSV serology.

Neonatal Outcomes

Ten deaths occurred among infants during the follow-up period including five deaths among liveborn infants within the first 28 days. The neonatal mortality rate of 5/394 live births (13/1000; 95% CI, 5-30/1000 live births) is similar to the estimated rate of 18-19/1000 live births in South Africa for 2010-2011.[26,27] None of the infants who died were evaluated for neonatal herpes. Three stillborn infants were delivered from HIV-negative women, two of whom were HSV-2 seropositive and none of whom had HSV-2 shedding detected (Table 4). Five neonates who died were also born to HIV-negative women, three of whom where HSV-2 seropositive, and none of whom had HSV-2 shedding detected. Two infants died

after the neonatal period, one at day 35 and one at day 36 of life. Both were born to women coinfected with HIV and HSV-2, and both women had genital HSV shedding detected. Twenty-seven infants were identified as potentially exposed to HSV during delivery, as they were born to women with HSV shedding detected and were delivered vaginally or by cesarean section after prolonged rupture of membranes (>4 hours);[5,28,29] of these, two (7.4%) died during the follow-up period. The cause of death was pneumonia in one case and possible pneumonia in the other. It is uncertain if either infant was exposed to HSV during birth, however, as the genital swabs were obtained thirteen and five days, respectively, prior to the delivery date. For the other 25 potentially exposed neonates, 13 were healthy at the post-partum visit, six additional neonates were doing well on follow-up phone call (range, 10-21 days post-partum), and six were lost to follow-up.

Discussion

Our study demonstrates that prior HSV-2 infection is frequent among pregnant women in South Africa, as is genital HSV shedding during labor, especially among HIV-positive women. The shedding frequency is similar to women in the U.S. during labor, where genital HSV was detected in 30.8% of HIV-positive and 9.5% of HIV-negative women.[30,31] Our study suggests that the lack of published reports of neonatal herpes in South Africa is not due to infrequent exposure to HSV-2 during birth.

While we did not identify any cases of overt neonatal herpes, the study was not powered to estimate the frequency of neonatal herpes. It is unlikely that the two infants born vaginally to women with HSV-2 reactivation at enrollment (five and thirteen days prior to delivery) were infected with HSV. While pneumonia can be a feature of disseminated HSV infection, this typically occurs at day 10-12 of life.[2] A systematic evaluation of acutely ill neonates, with diagnostic testing by HSV PCR or viral culture, would be required to accurately estimate the rate of neonatal herpes in developing countries.

Specimens were collected at enrollment from women thought to be in early labor, but only half of women are known to have delivered within one day of genital swab collection. As cell-mediated immunity is impaired during pregnancy,[32] women experience an increased number of clinical recurrences from the first to third trimester.[33] Our results were similar when the analysis was restricted to women who delivered within one day of genital swab collection, however. While the delay between swab collection and delivery does not affect the determination of shedding frequency, our ability to determine which

neonates were at greatest risk for neonatal herpes due to contact with HSV-2 during delivery was limited. In immunocompetent persons, with daily swab collection, subclinical shedding episodes last a median duration of only 2 days (IQR, 1.0-3.5).[15] Studies with more frequent sampling (four times daily) in HIV-positive and immunocompetent persons have suggested that half of episodes may be <12 hours in duration.[16,34] To more accurately assess which neonates are at greatest risk for neonatal herpes, genital swabs for HSV PCR would need to be collected closer to the time of delivery.

Although 41% of participants were HSV-2 seronegative at enrollment, we did not detect any episodes of HSV-2 acquisition in late pregnancy: no women seroconverted and HSV-2 shedding was detected in no HSV-2 seronegative women. In neighboring Zimbabwe, the incidence of HSV-2 seroconversion was 1.8% between 36 weeks of gestation and 6 weeks post-partum[35] and among HIV-positive women, 17.3% seroconverted between delivery and 6 weeks post-partum.[36] These rates are considerably higher than the U.S., where between the first prenatal visit and delivery (median interval of 6.5 months) the HSV seroconversion rate is 1.3%, with 1.0% developing HSV-2 antibodies.[7] Although we have complete data on genital HSV-2 shedding in HSV-2 seronegative women, the expected shedding rate with recent HSV-2 acquisition is less than 50%,[37] so first episode infections in late pregnancy may not have been captured by detection of genital HSV shedding alone. Our incomplete follow-up, however, limits the number of women evaluated for seroconversion. With an estimated HSV-2 incidence of 5-10%,[13] only 1.3 to 2.5% of the study population would be expected to have acquired HSV-2 in the past three months. While no participants seroconverted, the 95% confidence interval (0-3.2%) includes the percentage of women expected to seroconvert if annual HSV-2 incidence was 10% and remained unchanged in late pregnancy.

Despite the limitations in detecting HSV-2 acquisition in late pregnancy, this study nevertheless demonstrates a high rate of genital HSV-2 shedding during labor in HSV-2 seropositive women. While the risk of neonatal transmission for women with HSV reactivation is thought to be <1%[3] due to protection by maternal neutralizing antibodies, 20-50% of neonatal herpes cases in the U.S. and Europe are associated with HSV-2 reactivation[6-8] due to the high prevalence of HSV-2 relative to the low incidence of HSV-2 in late pregnancy. If the absence of published reports of neonatal herpes from Africa reflects decreased neonatal infection with HSV, as opposed to decreased identification and reporting, given the

high genital shedding rate that we have found among HSV-2 seropositive women, there may be differences in the transmissibility of HSV-2 to the neonate due to virologic, host, or iatrogenic (e.g. decreased use of invasive fetal monitoring devices) factors. Studies investigating genetic differences in HSV-2 strains as well as immunologic differences between South African and U.S. mother-infant pairs may provide insights into the pathogenesis of neonatal HSV.

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Table 1. Baseline participant characteristics

Characteristic	HIV-positive n=132 (34.1%)	HIV-negative n=255 (65.9%)	Total n=390
Age, mean (range), yrs	29 (18-40)	26 (18-44)	27 (18-44)
Gravidity, n (%)			
Primigravid	23 (17.6)	112 (44.8)	135 (35.4)
Multigravid	108 (82.4)	138 (55.2)	246 (64.6)
EGA at enrollment, mean (range), weeks	39 (32-44)	39 (30-42)	39 (30-44)
HSV serostatus by Western blot, n (%)			
HSV-1+ only	16 (12.1)	144 (56.5)[a]	161 (41.3)
HSV-2+ only	5 (3.8)	2 (0.8)	7 (1.8)
Both HSV-1+&HSV-2+	111 (84.1)	109 (42.8)	222 (56.9)
History of genital ulcers among HSV-2 seropositive, n (%)	16 (14.2)	6 (5.5)	22 (9.9)
Genital lesions present among HSV-2 seropositive, n (%)	3 (2.6)	4 (3.6)	7 (3.1)
CD4 count, median (range), cells/μL[b]	321 (18-1237)		
Antiretroviral therapy			
HAART[c,d]			
Any CD4 count	52/132 (39.4)		
CD4 ≤350 cells/μL	46/70 (65.7)		
CD4 ≤200 cells/μL	21/26 (80.8)		
Prophylaxis to prevent mother-to-child transmission	78 (59.1)		
Total receiving antiretroviral drugs[e]	131 (99.2)		

Abbreviations: EGA, estimated gestational age; HAART, highly active antiretroviral therapy (≥ three antiretroviral drugs).
Missing data: HIV-status (n=3), gravidity (n=9), EGA (n=4), history of genital ulcers among HSV-2 seropositive (n=7), CD4 count (n=6).
Percentages determined by excluding those with missing data from the denominator. [a]Includes one participant with HSV-1 and atypical HSV-2 bands on Western blot at baseline for whom we did not obtain a post-partum Western blot. [b]CD4 count obtained a median of 99 days prior to enrollment (IQR, 57-129 days). [c]Denominator is the number of participants with CD4 count within range specified. [d]In April 2010, one month prior to initiation of this study, South African National Department of Health guidelines were revised to recommend HAART initiation for all pregnant women with a CD4 count ≤350 cells/μL;[38] previous guidelines recommended HAART initiation for a CD4 count ≤200 cells/μL or WHO stage IV disease. [e]Total receiving antiretroviral drugs does not equal those receiving HAART + those receiving prophylaxis to prevent mother-to-child transmission as the only antiretrovirals recorded for one participant were zidovudine and lamivudine.

Table 2. Risk factors for HSV-2 infection and genital HSV-2 shedding in labor among HSV-2 seropositive women

	Genital HSV-2 shedding among HSV-2 seropositive women[a,b]				HSV-2 seropositivity					
Variable	Genital HSV-2 present n=39 (17.2%) n (%)	Genital HSV-2 not present n=188 (82.8%) n (%)	Unadjusted RR (95%CI)[c,d]	*P*-value	HSV-2 seropositive n=229 (58.7%) n (%)	HSV-2 seronegative n=161 (41.3%) n (%)	Unadjusted RR (95%CI)[c]	*P*-value	Adjusted RR (95%CI)[c,e]	*P*-value
HIV status										
HIV-positive	26 (22.6)	89 (77.4)	1.91 (1.04, 3.53)	0.038	116 (87.9)	16 (12.1)	2.02 (1.73, 2.35)	<0.001	1.69 (1.45, 1.96)	<0.001
HIV-negative	13 (11.8)	97 (88.2)	1.00 (ref.)		111 (43.5)	144 (56.5)	1.00 (ref.)		1.00 (ref.)	
Age, per each 10 y increase			0.68 (0.41, 1.13)	0.14			1.62 (1.45, 1.82)	<0.001	1.28 (1.12, 1.46)	<0.001
Gravidity										
Primigravid	10 (24)	32 (76)	1.0 (ref.)		42 (31.1)	93 (68.9)	1.00 (ref.)		1.00 (ref.)	
Multigravid	28 (15.5)	153 (84.5)	0.65 (0.34, 1.23)	0.19	183 (74.4)	63 (25.6)	2.39 (1.84, 3.11)	<0.001	1.68 (1.26, 2.25)	<0.001
Among HIV+ patients:										
CD4 count, cells/µL										
<200	7 (28)	18 (72)	1.3 (0.5, 3.5)	0.62	25 (96)	1 (4)	1.1 (0.9, 1.3)	0.18		
200-349	7 (20)	28 (80)	0.9 (0.3, 2.6)	0.87	36 (84)	7 (16)	1.0 (0.8, 1.2)	0.87		
350-499	6 (23)	20 (77)	1.1 (0.4, 3.0)	0.91	26 (87)	4 (13)	1.0 (0.8, 1.3)	0.87		
≥500	5 (22)	18 (78)	1.0 (ref.)		23 (85)	4 (15)	1.0 (ref.)			
Receiving HAART										
Yes	12 (26)	34 (74)	1.3 (0.7, 2.5)	0.47	47 (90)	5 (10)	1.0 (0.9, 1.2)	0.46		
No	14 (20)	55 (80)	1.0 (ref.)		69 (86)	11 (14)	1.0 (ref.)			
HAART regimen containing tenofovir										
Yes	4 (22)	14 (78)	0.8 (0.3, 2.2)	0.64	19 (86)	3 (14)	0.9 (0.8, 1.1)	0.43		
No	8 (29)	20 (71)	1.0 (ref.)		28 (93)	2 (7)	1.0 (ref.)			

Abbreviations: RR, relative risk; HAART, highly active antiretroviral therapy (≥ three antiretroviral drugs).
Missing data: Genital HSV DNA (n=2), HIV-status (n=3), gravidity (n=9), CD4 count (n=6). Percentages determined by excluding those with missing data from the denominator. [a]HSV DNA was typed as HSV-2 in all cases. [b]Genital HSV-2 DNA was not detected in any HSV-2 seronegative women. [c]As determined by Poisson regression. [d]Only HIV-status remained significantly associated with HSV-2 shedding after inclusion of maternal age and history of prior pregnancy in a multivariate model and backwards elimination of non-significant (P<0.05) variables. [e]Adjusted for HIV-status, age, and prior pregnancy.

Table 3. Association of HIV-status with clinical manifestations of HSV-2 and quantity of HSV-2 shed among HSV-2 seropositive women

	HIV-positive n=116	HIV-negative n=111	Unadjusted RR (95% CI)[a]	*P*-value
History of genital ulcers, n (%)	16 (14.2)	6 (5.5)	2.57 (1.04, 6.34)	0.040
No. episodes of genital ulcers in the past year among women reporting a prior history, mean (range)	2 (0-12)	9 (1-24)		0.11
Genital lesions identified at enrollment, n (%)	3 (2.6)	4 (3.6)	0.72 (0.16, 3.14)	0.66
Quantity of HSV-2 DNA when detected, mean (range), log_{10}copies/mL[b]	4.57 (2.17-7.44)	4.42 (2.29-7.05)		0.80

Abbreviations: RR, relative risk for outcomes by HIV-status.

Missing data: HSV-2+ women with history of genital ulcers (HIV+: n=3; HIV-: n=2), No. episodes of genital ulcers in the past year (HIV+: n=3). Percentages determined by excluding those with missing data from the denominator. [a]As determined by Poisson regression. [b]The mean quantity of HSV-2 DNA for the two participants with genital lesions and HSV shedding was 6.41 (range, 5.83-6.98) compared with 4.42 (range, 2.17-7.44) log_{10}copies/mL in the 37 women with subclinical shedding.

Table 4. Deaths known to have occurred among infants during the follow-up period, n=10

Maternal age (years)	Maternal comorbidities[a]	EGA (weeks)	Birthweight (g)	Delivery route	Maternal HSV-2 serostatus at delivery	Maternal genital HSV-2 (log_{10} copies/mL)	Days between delivery and swab collection	Age at death (days)	Cause of death
20	HIV (CD4 229) ARVs: 3TC, TDF, NVP	40	3150	Vaginal	Positive	2.28	13	35	Pneumonia
30	HIV (CD4 489) ARV: AZT	39	3130	Vaginal	Positive	2.56	5	36	Possible pneumonia
42	Gestational diabetes	37	2200	Vaginal	Positive	ND	50	Stillborn	
33	None	41	3971	Vaginal	Positive	ND	4	Stillborn	
22	None	37		Vaginal	Negative[b]	ND	0	Stillborn, twin survived	
23	None	42	3930	Vaginal	Positive	ND	1	0	Perinatal asphyxia
24	Epilepsy, treated with valproic acid	42	2325	Vaginal	Positive	ND	2	1	Respiratory failure
38	Pregnancy-induced hypertension	41	3230	Vaginal	Negative[b]	ND	2	4	Perinatal asphyxia
33	Hypertension	38	2220	Cesarean section	Positive	ND	4	8	Details not available
22	None	37	2780	Cesarean section	Negative[b]	ND	1	26	Congenital heart disease, pneumonia

Abbreviations: EGA, estimated gestational age; ARVs, antiretrovirals; 3TC, lamivudine; TDF, tenofovir; NVP, nevirapine; AZT, zidovudine. ND, not detected. [a]All ten women had a non-reactive rapid plasma reagin (RPR) during the prenatal period.
[b]Post-partum HSV Western blot not available.

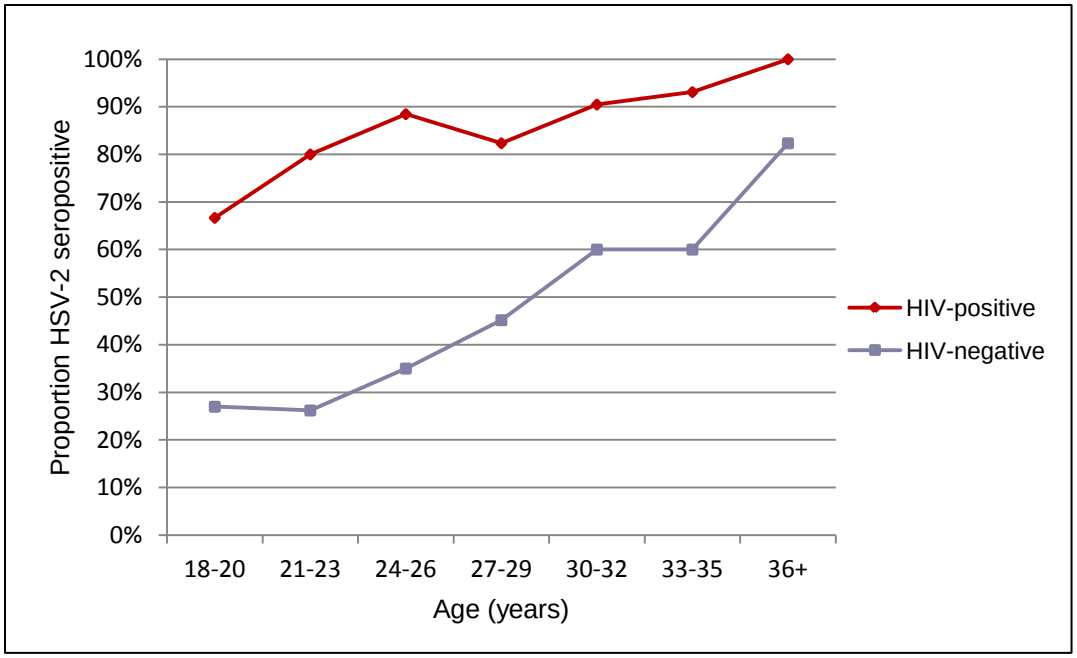

Figure. HSV-2 seroprevalence per age group, stratified by HIV status.