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A Prospective Study of Risk Factors for Herpes Simplex Virus Type 2 Acquisition among High-Risk HIV-1 Seronegative Kenyan Women

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Abstract

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Vrasha Chohan, is the corresponding author. She conducted or oversaw all laboratory testing of HSV-2, and other STIs for the study. She led the drafting of the manuscript.
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Ludo Lavreys, oversaw and contributed to collection of the clinical data and samples for the study. He contributed to drafting of the manuscript and has approved the final draft.
Kishorchandra Mandaliya, supervised laboratory work and quality assurance for the study. He contributed to drafting of the manuscript and has approved the final draft.
Jeckoniah O. Ndinya-Achola, contributed to the establishment of the cohort, including development of the core clinical and laboratory procedures. He helped to oversee both clinical work and laboratory procedures for the current study. He contributed to drafting of the manuscript and has approved the final draft.
Walter Jaoko, contributed to the study design and provided oversight for both the clinical and laboratory aspects of the study. He contributed to drafting of the manuscript and has approved the final draft.
Julie Overbaugh, contributed to the establishment of the cohort and the design of the current study. She secured the funding for HSV testing in the cohort. Dr. Overbaugh contributed to drafting the manuscript and approved the final draft.
R. Scott McClelland, contributed to the design and core procedures of the cohort. He also helped to develop the design of the current study and interpretation of the findings. He oversaw manuscript preparation and has approved the final draft.

Objectives—Several studies have demonstrated an association between herpes simplex virus type 2 (HSV-2) and human immunodeficiency virus type 1 (HIV-1), but limited data on risk factors for HSV-2 acquisition are available. The objective of this analysis was to determine the incidence and risk factors for HSV-2 acquisition among HIV-1-seronegative Kenyan female sex workers.

Methods—Between February 1993 and December 2006, HIV-1-seronegative women attending a municipal sexually transmitted infections (STI) clinic were invited to enroll in a prospective cohort study. Screening for HIV-1 and STIs were done at monthly follow-up visits. Archived blood samples were tested for HSV-2.

Results—Of 1527 HIV-1-seronegative women enrolled, 302 (20%) were HSV-2-seronegative at baseline, of whom, 297 had at least one follow-up visit. HSV-2 incidence was high (23 cases/100 person-years; 115 cases). In multivariate analysis, HSV-2 was significantly associated with more recent entry into sex work, workplace, and higher number of sex partners per week. Condom use was protective, although this was statistically significant only for the intermediate strata (25–75% condom use, HR 0.43, p=0.05). There were statistical trends for bacterial vaginosis to increase HSV-2 risk (HR 1.56, p=0.07) and for oral contraceptive use to decrease risk (HR 0.50, p=0.08). The 23% annual HSV-2 incidence in this study is among the highest reported anywhere in the world.

Conclusions—Women were at increased risk if they had recently entered sex work, had a higher number of sex partners, or worked in bars. HSV-2 risk reduction interventions are urgently needed among high-risk African women.

Keywords

herpes simplex virus type 2; incidence; risk factors; HIV-1 seronegative; female sex workers

Introduction

The etiology of genital ulcer disease (GUD) has evolved over the last decade, with herpes simplex virus type-2 (HSV-2) replacing syphilis and chancroid as the most frequent cause.[1] In sub-Saharan Africa, the region most affected by HIV-1, HSV-2 seroprevalence rates as high as 40–80% have been observed.[2] High rates of herpes may contribute substantially to the spread of HIV-1, since HSV-2 infection has been associated with increased risk for HIV-1 acquisition.[3] However, there are limited data about risk factors for HSV-2 acquisition in African populations.

Female sex workers in Africa have been recognized as a particularly vulnerable population for HIV-1 infection since early in the epidemic, [4] and sex workers continue to have an important effect on population-level HIV-1 transmission in Africa.[5] Our objective was to determine the incidence and risk factors for HSV-2 acquisition among HIV-1-seronegative Kenyan sex workers.

Methods

In February 1993, a prospective cohort study of women attending a municipal sex worker clinic was initiated in Mombasa, Kenya. One of the primary aims of this cohort has been to determine risk factors for HIV-1 and STIs. Detailed procedures have been described previously.[6] Briefly, women were offered confidential HIV-1 counseling and testing, and HIV-1-seronegative women were invited to enroll. At enrollment and monthly follow-up visits, women completed face-to-face interviews using standardized questionnaires to ascertain medical, gynecological, and sexual history. A physical examination was performed, including a pelvic examination with collection of specimens for laboratory diagnosis of STIs. The

presence of genital ulcers was recorded. A blood sample was obtained for HIV-1 serological testing, and aliquots from each sample were archived at −80°C.

Women with STI symptoms were provided with syndromic management in accordance with Kenya Ministry of Health guidelines. Women were invited to return one week after each monthly visit for laboratory results, and additional treatment was provided for infections detected on laboratory testing that had not been recognized at the time of the examination. At each visit, HIV-1 risk reduction counseling and free condoms were provided. The ethical review boards at the University of Washington, Kenyatta National Hospital and the Fred Hutchinson Cancer Research Center approved the study. All participants provided informed consent.

Screening for HIV-1 infection was performed by ELISA (Detect HIV Biochem ImmunoSystems). Positive samples were confirmed using a second ELISA (Recombigen, Cambridge Biotech or Vironostika, Biomerieux). Serological testing for HSV-2 was performed on archived samples using a type-specific HSV-2 gG based ELISA (HerpeSelect 2, Focus Diagnostics). An index value of >1.1 (the ratio of the optical density [OD] of the sample to the OD of the standard calibrator) was considered to be positive for HSV-2 as per the manufacturer's instructions. While early data suggested that index values between 1.1 and 3.5 had reduced specificity for defining HSV-2 serostatus in African specimens, [7] a recent study comparing the HerpeSelect and Kalon assays showed that low-positive index values generally correspond to true HSV-2 infection, particularly in cases with recent HSV-2 acquisition in the settings of prior HSV-2 seronegativity.[8] Thus, we felt that a cutoff index value of 1.1 was appropriate for this study of HSV-2 acquisition.

A staged protocol was used in testing samples for HSV-2. First, a sample from each participant's enrollment visit was tested. Next, women with negative enrollment results had their last follow-up samples tested to define their HSV-2 serostatus at the end of the study interval. Finally, for those who were HSV-2 seropositive at their last follow-up visit, intervening samples were tested to determine the timing of seroconversion. The archived enrollment samples were serum and follow-up samples were plasma. The HSV-2 ELISA used in this study provides similar results with both sample types.[9]

Vaginal wet preparations were examined at 40× magnification for the presence of *Trichomonas vaginalis* and yeast. Bacterial vaginosis (BV) was diagnosed based on Gram stain criteria. [10] Gram stained cervical secretions were examined microscopically and cervicitis was defined as an average of ≥30 polymorphonuclear leukocytes in three non-adjacent oil immersion fields. Endocervical secretions were inoculated on modified Thayer-Martin medium for isolation of *Neisseria gonorrhoeae*.

Data from February 1993 through December 2006 were used. Women who were HSV-2-seronegative at baseline were selected. All visits at which HSV-2 serostatus could be determined were included. Analyses were performed using SPSS version 10 (SPSS) and S-Plus 2000 (Mathsoft). Women who seroconverted to HSV-2 were censored at that visit. For time-to event analyses, HSV-2 seroconversion was considered to have occurred on the date HSV-2 seropositivity was detected. Since our aim was to assess risk factors for HSV-2 acquisition among HIV-1-seronegative women, those who became infected with HIV-1 were censored at the time of HIV-1 seroconversion.

Several potential risk factors for HSV-2 acquisition were analyzed. Information concerning educational level, parity, workplace (bar vs. nightclub), alcohol and tobacco use, and vaginal washing practices were based on data collected at enrollment. Time-dependent modeling was used for other covariates including age, duration of sex work, contraceptive method and the presence of other genital tract infections (gonorrhea, cervicitis, BV, vaginal candidiasis,

trichomoniasis, and genital ulcer disease). As we have done previously for analyses of the effect of genital infections on risk of HIV-1 seroconversion, [3] we assumed an effect window of 60 days to capture the influence of genital tract infections on HSV-2 seroconversion risk. Two measures of sexual behavior, number of sex partners per week and percentage condom use were analyzed as time-dependent covariates. To capture usual behavior over time, averages were calculated for these variables for each year of follow-up.[11] We modeled condom use in three categories (<25%, 25–75%, and >75%) to remain consistent with prior studies.[12]

Cox proportional hazard models were used to calculate univariate and multivariate hazard ratios (HRs) and 95% confidence intervals (CIs) for potential risk factors related to HSV-2 acquisition. The final multivariate model was constructed using reverse stepwise selection of variables. Starting with a model including all covariates from the univariate analyses, one variable at a time was excluded. The variable removed was selected based on having the P-value closest to 1.0. This process was repeated until all variables remaining in the model had P-values ≤0.10. For variables with multiple categories (e.g. contraceptive method, each method as a separate category versus none), the lowest P-value for any category was considered when selecting variables for exclusion. Genital ulcer disease was not included in the multivariate model, as this was felt to be a consequence of HSV-2 infection rather than a risk factor.

Results

A total of 1786 HIV-1-seronegative women were enrolled between February 1993 and December 2006, of whom 1527 (85.5%) returned for at least 1 follow-up visit. For determination of HSV-2 serostatus, 1506 (98.6%) samples were available at enrollment, 302 (20.1 %) from women who were initially HSV-2 seronegative. Of these, 297 (98.3%) had follow-up samples available for HSV-2 testing and served as the study population for this analysis. Baseline characteristics are provided in Table 1. The median duration of follow-up was 335 days (interquartile range [IQR] 92-862), reflecting a total of 499 person-years of follow-up over 2819 visits. The median time between visits was 33 days [IQR 28-48]. One hundred fifteen women seroconverted to HSV-2 (incidence 23 cases/100 person-years). There were 10 women who experienced HSV-2 and HIV-1 seroconversion at the same visit.

In univariate analyses, recent entry into sex work, heavier alcohol use (>7 drinks per week), and BV were significantly associated with increased risk for HSV-2 (Table 2). More frequent condom use was protective, although this association was only statistically significant for the 25–75% condom use category. Acquisition of HSV-2 was associated with >10-fold increase in the risk of GUD (HR 10.40, 95% CI 4.57–23.80, p<0.001).

In the multivariate analyses, more recent entry into sex work remained significantly associated with increased HSV-2 risk. The association between condom use and reduced risk for HSV-2 also remained essentially unchanged, with significantly lower HSV-2 risk in the 25–75% condom use group. After adjustment for potential confounding factors in the multivariate model, higher number of sexual partners and bar work both achieved statistically significant associations with HSV-2 acquisition. There were statistical trends for increased risk of HSV-2 among women with BV (P=0.07), and for decreased risk of HSV-2 among women using oral contraceptive pills (P=0.08).

Discussion

Nearly 80% of the participants in this cohort of women at high risk for HIV-1 were seropositive for HSV-2 upon entry into the study. Among those who were initially seronegative, the rate of new HSV-2 infections was 23% per year. To our knowledge this is among the highest incidence rates for HSV-2 observed anywhere in the world.[13,14] Recent entry into sex work, bar work

(as opposed to nightclub work), higher number of sexual partners, and less frequent condom use were associated with elevated HSV-2 risk.

Our findings are comparable to a recent study of female bar and hotel workers in Tanzania.[13] That study, found an overall HSV-2 incidence of 14.2 cases/100 person-years. There was increased risk of HSV-2 acquisition in women who reported sexual debut at a younger age.[13] The high incidence of HSV-2 in these cohorts of East African women suggests an urgent need for interventions aimed at delaying sexual debut, encouraging fewer partners after initiation of sex, and promoting correct and consistent condom use.

In this population of female sex workers from Mombasa, we found a higher risk of acquiring HSV-2 among women working in bars as opposed to nightclubs. This is consistent with our previously reported findings of risk factors for HIV-1 in this cohort.[6] Most of the women work in bars, and supplement their income as barmaids with transactional sex. As such, they tend to have relatively lower numbers of sex partners. While the specific workplace association is unlikely to be widely generalizable, it highlights the fact that network-level factors may influence STI risk in a way that is not captured by adjustment for individual-level sexual risk behaviors such as number of partners, frequency of intercourse, and condom use.

We found that condom use reduced the risk of HSV-2 acquisition, but this association was statistically significant only for the 25%–75% condom use category. Surprisingly, the protective effect was smaller and was not statistically significant for the >75% condom use category. Given the use of participants' self reported behavior, the highest category of condom use may represent over-reporting due to social desirability bias. In addition, women with the highest reported condom use may have more sex partners or may be exposed to partners who are at higher risk for transmitting HSV-2. Nonetheless, it is encouraging to see that condom use appeared to provide some reduction in the risk of HSV-2 in this cohort.

In the present study, BV was associated with a significantly increased risk for HSV-2 acquisition in unadjusted analyses, although this was reduced to a statistical trend in the multivariate model. Prior studies have found an association between BV and HSV-2.[15,16] The relatively small number of HSV-2 seroconversions in our cohort may have limited our ability to achieve statistical significance for this modest association. Oral contraceptive use was associated with a 50% decreased risk for HSV-2 acquisition in multivariate analysis, although this did not achieve statistical significance. Few data have been reported concerning the effect of hormonal contraception on HSV-2 risk, although interactions between contraceptive use, HSV-2, and HIV-1 risk have been proposed.[3] Future studies should continue to explore the relationship between contraception and HSV-2 acquisition.

Ultimately, a safe and effective vaccine would provide the most effective means of reducing women's risk for acquiring HSV-2. Until such a vaccine is available, studies that help us to understand risk factors for HSV-2 such as higher partner number, lack of condom use, and potentially BV may help to inform the development of intervention strategies to reduce HSV-2 transmission.

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Table 1

Baseline Characteristics of HSV-2-Seronegative Female Sex Workers in Mombasa, Kenya (N=297)

Characteristic	Median (IQR) or number (%)
Age, years	23 (20–26)
Duration of sex work, years	0.4 (0–1)
Education, years	8 (7–11)
Bar work	180 (60.6%)
Tobacco use	55 (18.5%)
Alcohol use	205 (69.0%)
Vaginal cleansing	
Water	70 (23.6%)
Soap/other	208 (70.0%)
Sex partners/week	1 (1–2)
Condom use (during past working week)[1]	
<25%	60 (20.2%)
25–75%	21 (7.1%)
>75%	207 (69.7%)
abstinent	8 (2.7%)
Number of pregnancies[1]	
0	90 (30.3%)
1	119 (40.1%)
≥2	87 (29.3%)
Hormonal contraceptive use	
Oral contraceptive pills	45 (16.2%)
Depot medroxyprogesterone acetate/Norplant	56 (18.9%)
Genital tract conditions	
Genital ulcer disease	3 (1.0%)
Neisseria gonorrhoeae	7 (2.4%)
Cervicitis	36 (12.1%)
Bacterial vaginosis	86 (29.2%)
Vaginal candidiasis	54 (18.2%)
Trichomonas vaginalis	19 (6.4%)

Note. IQR, interquartile range

[1] N=296

Table 2

Correlates of Incident HSV-2 among Female Sex Workers in Mombasa, Kenya

Characteristic	Univariate HR (95% CI)	P-value	Multivariate aHR (95% CI)	P-value
Age, years				
<25	1.0			
25–34	0.72 (0.48–1.07)	0.1		
≥35	0.41 (0.17–1.03)	0.06		
Duration of sex work, years				
≤1	1.0		1.0	
2–4	0.59 (0.35–1.01)	0.05	0.57 (0.33–1.03)	0.06
≥5	0.12(0.05–0.29)	<0.001	0.07 (0.02–0.24)	<0.001
Education ≤8 years	1.16 (0.80–1.67)	0.4		
Bar work (vs. night club)	1.23 (0.85–1.78)	0.3	1.72 (1.08–2.74)	0.02
Tobacco (yes/no)	1.09 (0.71–1.66)	0.7		
Alcohol				
None	1.0			
1–7 drinks/week	0.86 (0.55–1.35)	0.5		
>7 drinks/week	1.55 (1.00–2.38)	0.05		
Vaginal cleansing				
None	1.0			
Water	0.88 (0.39–2.00)	0.8		
Soap/other[1]	1.01 (0.48–2.16)	1.0		
Sex partners per week				
<1	1.0		1.0	
1–2	1.42 (0.96–2.10)	0.08	2.37 (1.36–4.14)	0.002
>2	1.52 (0.86–2.68)	0.2	2.70 (1.25–5.84)	0.01
Condom use				
<25%	1.0		1.0	
25–75%	0.46 (0.21–1.00)	0.05	0.43 (0.19–0.99)	0.05
>75%	0.74 (0.43–1.28)	0.3	0.70 (0.38–1.29)	0.3
Number of pregnancies				
0	1.0			
1	1.14 (0.74–1.77)	0.6		
≥2	1.09 (0.66–1.80)	0.7		
Hormonal contraceptive use				
None	1.0		1.0	
OCP	0.61 (0.34–1.08)	0.09	0.50 (0.23–1.08)	0.08
DMPA or Norplant	0.95 (0.60–1.50)	0.8	0.92 (0.53–1.61)	0.8
Genital tract conditions				
Neisseria gonorrhoeae	1.25 (0.48–3.21)	0.7		
Cervicitis	1.44 (0.88–2.35)	0.2		
Bacterial vaginosis	1.70 (1.17–2.47)	0.006	1.56 (0.96–2.55)	0.07

Characteristic	Univariate HR (95% CI)	P-value	Multivariate aHR (95% CI)	P-value
Vaginal candidiasis	1.10 0.72–1.70)	0.7		
Trichomonas vaginalis	1.65 (0.87–3.13)	0.1		

Note: HR, hazard ratio; aHR adjusted hazard ratio; OCP, oral contraceptive pills; DMPA, depot medroxyprogesterone acetate

[1] Five (1.7%) women reported using detergent, 13 (4.4%) reported using antiseptic, and 95 (32.0%) reported using "other" substances for vaginal washing.