

Sero-prevalence of herpes simplex type 2 virus (HSV-2) and HIV infection in Kampala, Uganda

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Abstract

Background:

Prevalence of herpes simplex type 2 virus (HSV-2) is high worldwide. Previous studies in Uganda were rural or in women. We estimated age and sex-specific sero- prevalence of HSV-2 in Kampala, Uganda.

Methods: Using two-stage random sampling stratified on population density, a survey of persons 15-65 years was conducted. Type-specific serological tests for HSV-2, HSV-1(HerpeSelect2 and 1 ELISA), HIV (Rapid tests and ELISA), syphilis (RPR and TPHA) were done. Additional prevalence analysis included post-stratification weighting on the Uganda 2002 Census gender distribution.

Results: Among 1124 persons, HSV-2 prevalence was 58% (95% CI: 55, 60), HSV-1; 98% (95% CI: 97.6, 99.1), HIV; 17.7% (95% CI: 14.8, 19.2) and syphilis; 1.7% (95% CI: 1.4, 1.9). Weighted HSV-2 prevalence was 53.8% (Women; 63.8%, men; 43.2%), similar to unweighted data. Weighted HIV prevalence was 20.7% in women, 8.6% in men. Of 165 HIV infected persons, 85.4% had HSV-2. Risk factors for HSV-2 were being a woman (OR 2.0; 95% CI: 1.42, 2.78), age (OR 3.3; 95% CI: 2.43, 4.53), education (OR 1.70; 95% CI: 1.34, 2.34) and HIV (OR 4.5; 95% CI: 2.70, 7.50).

Conclusion: Prevalence of HSV-2 and HIV was high especially in women. Syphilis was rare. Awareness of herpes was low. Interventions in young people are needed.

Keywords: HSV-2, HIV, Kampala Uganda

DOI: <http://dx.doi.org/10.4314/ahs.v14i4.2>

Introduction

Infection with herpes simplex virus type 1 and 2 (HSV-1 and 2) is common worldwide¹. Estimates of HSV-2 prevalence in Africa are high; 78% in women and 45% in men ². No vaccine exists to prevent infection. HSV-2 infection is closely correlated to and fuels the HIV epidemic in sub-Saharan Africa and elsewhere^{3, 4, 5}. Studies to disrupt this synergy have been unsuccessful⁶. HSV-2 remains an important sexually transmitted disease (STD); inherently and because of its synergy with HIV.

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Previous studies to assess the prevalence of HSV-2 in Uganda were in rural settings⁷. At the time this study was conducted, there was limited population data on prevalence of HSV-2 infection in Uganda's urban areas. Recent studies in Uganda show high prevalence in women^{8, 9}, pregnant women and clinical trial populations ¹⁰ but population estimates of HSV-2 infection in urban Uganda are few.

Materials and Methods

Study design and procedures

From February 2004 to June 2004, we conducted a study evaluating the prevalence of HSV-2 among residents of Kawempe Division (one of five divisions in urban Kampala), Uganda. Using two-stage stratified random sampling weighted on population density, we randomly selected 7 of 18 parishes in Kawempe and stratified these 7 on population density. Using detailed maps of all roads in the area from the Uganda Bureau of Statistics, we numbered the mapped road junctions in each parish. The road junctions were then randomly

selected with replacement from a covered box as points for starting data collection each morning. The direction research assistants took was selected with replacement from a box with four directions. We approached people in every third housing unit and one consenting person was chosen without replacement in every third housing unit to enroll.

Enrolled participants were 15 to 65 years old, had lived in the area for at least two weeks, and able to give written informed consent or assent to the study. Participants consented to HSV-2 testing but could opt out of HIV testing. Participants received pre and post test HIV counseling and if HIV positive, given referral for care to Mulago National Referral Hospital or clinic of their choice. HSV-2 positive persons with symptoms were referred to the STD clinic, reviewed and provided with episodic acyclovir treatment during the study. Trained research assistants obtained consent from eligible persons prior to confidential interviews and blood draws. We used standardized pre-tested questionnaires to collect data on socio-demographics, sexual history, history of symptoms related to genital ulcer disease and other STDs. Return visits over weekends and evenings were conducted but did not change distribution of men enrolled.

A contact address was collected. Consenting participants provided a sample of blood (5ml) for laboratory testing.

We tested sera for HSV-1 and 2 using type-specific serological tests (HerpeSelect 1 and 2 ELISA.), Focus Technologies, California)¹¹. We considered index values of 3.5 and above to indicate HSV-2 infection. We explored the 2.2 cut off and 1.1 cut off to describe potential lower limits for prevalence; the manufacturer's cut off was considered diagnostic for HSV-1 infection. HIV was tested using HIV ELISA (HIV ELISA Vironostika HIV Uni-form II Ag/Ab (BioMerieux Boxtel, Netherlands). Persons with a positive HIV ELISA test received a rapid test (Capillus HIV-1/HIV-2, Trinity Biotech, USA) for confirmation. For those with discordant results on these two tests, we performed a third rapid HIV test (Abbot Determine, Abbott Laboratories Abbott Park IL, USA) as a tie breaker. Persons testing positive on two of the tests were considered HIV infected. We tested for syphilis using the RPR Test (Human GmbH Wiesbaden, Germany) and confirmed with TPHA (Syphilis TPHA liquid Test (Human GmbH Wiesbaden, Germany). The study was reviewed and

approved by University Hospitals of Cleveland Ethical Review Board, the Joint Clinical Research Center (JCRC) Ethical Review Board and the Uganda National Council of Science and Technology.

We calculated the proportion of persons with HSV-2 infection with 95% confidence intervals using the 3.5 cut off. We also conducted sensitivity analyses for HSV-2 prevalence with the FDA approved cut off (1.1 and above) and a 2.2 cut off suggested in an earlier paper with colleagues¹². The final reported results are based on the 3.5 cut off to increase specificity^{13, 14}. We calculated weighted proportions for HSV-2 and HIV infection among women and men to mitigate potential bias due to skewed response from women (70% in study compared to a 51% population distribution)¹⁵. We calculated the proportion of people with HSV-1 and syphilis infection with 95% confidence intervals. Chi-square tests, F-tests of significance and univariate logistic regression were used to assess socio-demographic factors associated with odds of HSV-2 infection. To assess confounding and interaction, we built multivariate logistic models to include factors significant at $p \leq 0.05$ on univariate analysis. The model fit was evaluated using Hosmer-Lemeshow goodness of fit tests. As a sensitivity analysis, we repeated our regression with the HerpeSelect 2 manufacturer's cut off for positivity of 1.1 index value and the 2.2 index value but this did not appreciably change the results (data not shown). Data was analyzed using PASW 10.0 (PASW Inc, Chicago IL) and SAS version 9.1 software (SAS Institute Inc. Cary, North Carolina).

A sample size was generated for each selected stratum proportionate to population size. We calculated a sample size of 1073 persons based on an overall HSV-2 prevalence of 54%, and for specific age groups 15-19, 25-29 and greater than 40 years from prevalence studies on HSV-2 in rural Uganda with 5% error and after sample allocation and weighting for population density^{16 17}.

Results

We enrolled 1124 persons who provided questionnaire data and blood samples for the HSV-2 and HSV-1 antibody test. 971 persons (85.5%) consented to HIV testing and all but 11 (1%) consented to receiving HIV results. Enrolled participants included 786 (70 %) women and 338 (30 %) men. Median age was 26 years (Interquartile range [IQR] 22 to 33 years). Men and women enrolled were similar as regards age ($p=0.44$).

The most common occupation was small business followed by housewives (Table 1). Students made up 117 (10.4%) and 149 (13.3%) were unemployed. More than half were married (615, 54.7%) and in a stable partnership. Nearly half of participants had a primary education. Men had significantly higher education than women ($p<0.001$) and were more likely to have extra marital partners (30% vs 12%). Women were more likely to have an older sexual partner and to have had earlier sexual intercourse. Only 94 persons (8.4%) reported using condoms for every sex act in the past thirty days. Nearly half reported they used

Table 1 Unadjusted and Adjusted Models for Correlates of Prevalent HSV-2 in the Survey Population

VARIABLE	N	HSV-2 Positive n (%)	Unadjusted Odds Ratio (95%CI)	Adjusted Odds Ratio (95%CI)
Age				
≥25	546	233 (43)	1.00 (Ref)	
26+	578	417 (72)	3.47 (2.71 - 4.46)	3.30 (2.43-4.53)*
Age at first sex				
≥19 years	240	111 (46)	1.00 (Ref)	
<19 years	884	539 (61)	1.82 (1.36-2.42)	1.07 (0.85-1.36)
Gender				
Male	338	147 (43)	1.00 (Ref)	
Female	786	503 (64)	2.31 (1.78 - 2.91)	2.00 (1.42-2.78)*
Education				
> 7years	540	260 (48)	1.00 (Ref)	
≥7 years	583	390 (67)	2.17 (1.71 - 2.77)	1.70 (1.34-2.34)*
Relationship Type *				
Open	798	461 (58)	1.00 (Ref)	
One partner	316	182 (58)	1.1 (0.76-1.29)	1.05 (0.69-1.55)
Duration of Partnership				
> 2 years	601	396 (66)	1.00(Ref)	
≥2 years	324	145 (45)	0.42 (0.32-0.55)	0.82 (0.57-1.20)
Condom Use				
High Users	250	111	1.00 (Ref)	
Low users	767	474	1.93 (1.4-2.64)	1.34 (0.97-1.96)
HIV Status(n=971)				
Negative	806	424 (52.6)	1.00 (Ref)	
Positive	165	141 (85.4)	5.29 (3.3 - 8.3)	4.52 (2.73-7.49)*
Declined	153			

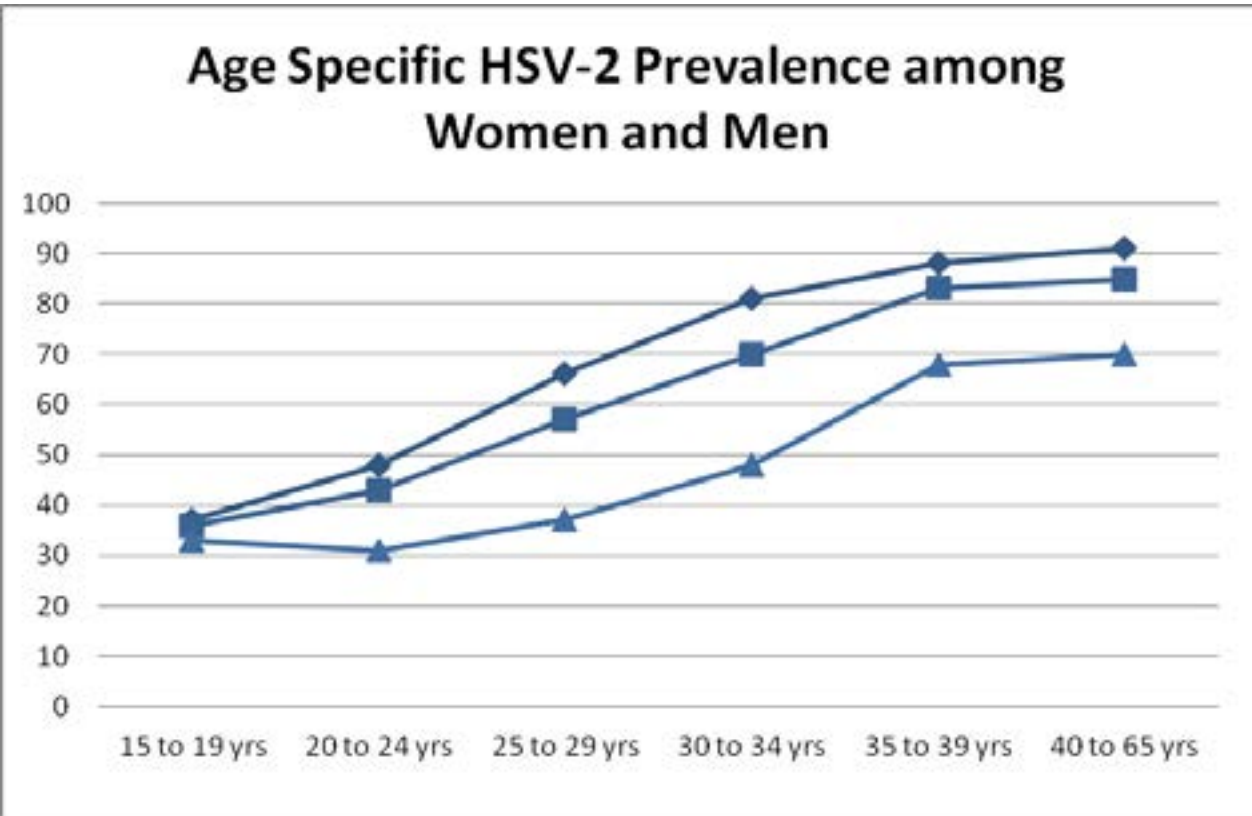
<10% missing data, * $p<0.05$

condoms rarely in the past thirty days and 52 (5%) had not used condoms in the past 30 days. Condom use was unusual with regular partners.

Of 1124 participants, 648 (58%, 95% CI: 55,60) tested HSV-2 positive using the HerpeSelect 3.5 cut off (75% and 61% tested positive using the 1.1 and 2.2 cut off respectively). Weighted analysis on gender distribu-

tion did not appreciably change the overall and gender specific prevalence (57.8 % overall ; 64.0% for women and 43.0% for men). Gender specific HSV-2 prevalence was similar in the weighted and the un-weighted data. Prevalence of HSV-2 increased with age (36% in the 15-19 year age group to 85% in the 40 and above age group). Women had significantly higher prevalence of HSV-2 compared to men ($p<0.0001$) and the differences increased with age (Figure 1).

Figure 1. Age Specific HSV-2 Prevalence by Gender



A total of 1106 persons (98 %, 95% CI: 97.6, 99.1) tested HSV-1 positive. Seventeen percent of people had HIV infection (95% CI: 14.80, 19.15). Men had significantly lower HIV prevalence (8.6%) than women (22%), ($p<0.001$). On weighting, HIV prevalence was 16% (95% CI: 15.87, 16.10), HIV prevalence for women was 19.9% and for men 8.4%, similar to the un-weighted data. Syphilis in this population was rare at 1.7% (95% CI: 1.40, 1.90).

On univariate analysis, women were more likely to have HSV-2 infection (OR 2.0,95% CI 1.42-2.78) compared to men. Age of participants, age when they first had sex , years of education, condom use with regular partner and HIV status were associated with HSV-2 prevalence (Table 1). Log odds of HSV-2 infection increased linearly with age ($p<0.001$). Among people who reported use of condoms with their regular partner, people who used condoms more often had lower odds of HSV-2 infection (OR 0.5; 95% CI: 0.48-0.64). There was no statistical evidence for two way interaction between gender and age group; (Breslow Day Test for Homogeneity of the Odds Ratio $p=0.11$, Mantel-Haenszl Common Odds Ratio (MH COR 3.43 ; 95% CI: 2.55, 4.62); between

age group and HIV status, (Breslow Day Test for Homogeneity of the Odds Ratio $p=0.73$, MH COR 5.18 ; 95%CI: 2.6,10.1) nor between gender and HIV status (Breslow Day Test for Homogeneity of the Odds Ratio $p=0.33$, (MH COR 5.59 ; 95% CI: 2.88,10.81) .

In the adjusted multivariable logistic model (Table 1), risk factors associated with HSV-2 infection were gender (OR 2.0; 95% CI: 1.42, 2.78), being older than 25 years (OR 3.3; 95% CI: 2.43, 4.53), education level (OR 1.70; 95% CI: 1.34, 2.34) for persons with less than7 years of education and HIV status (OR 4.5; 95% CI:2.70, 7.50). 141(14.5%) of all sample study participants were dually infected with HSV-2 and HIV. Of 165 persons infected with HIV, 85.4% also had HSV-2 infection. In contrast, among the HIV negative persons 52.6% (424) had HSV-2 infection. In total, 558 persons reported a history of genital ulcers.

Of the 648 people who tested HSV-2 positive, 301 (46.5%) reported history of ulcers and thought those ulcers were due to syphilis infection. However in this sample, only 19 people out of the 1124 (1.69%) tested positive for syphilis. Of these 15 (79%) had HSV-2 infection.

Discussion

In this population-based survey in Kampala, we found high prevalence of HSV-2 infection (58%), demonstrating a high burden of genital herpes in urban Uganda.

Prevalence among women 20 years and above was significantly higher than in men. However, there was similarity in HSV-2 prevalence in the 15 to 19 year age group for men and women. Prevalent HIV infection was high over all but higher among women. Syphilis was rare but many with history of genital ulcers who tested HSV-2 positive, reported they thought the ulcers were due to syphilis and only rarely due to herpes. Risk factors for prevalent HSV-2 infection included being a woman, older age, low condom use, younger age at first sex and presence of HIV infection similar to earlier studies.¹⁸ These associations were significant on univariate and multivariable analyses, again similar to several studies³. The report shows a high rate of HSV-2 co-infection among HIV infected persons.

To our knowledge, this is one of the few population studies on HSV-2 prevalence and associated risk factors in urban Uganda. Prevalence of HSV-2 was higher than in earlier rural population studies in Uganda (Rakai, Masaka)^{19,20}. The Uganda national sero-behavioral survey done in 2004-5 showed similar prevalence of HSV-2²¹ with a different serological test and low syphilis. Similar HSV-2 prevalence (30-80%) has been reported from other urban sub-Saharan locations. For example, in urban Zimbabwe HSV-2 prevalence was 68% and in Ndola, Zambia 55%.²² In contrast, population-based studies found substantially lower prevalence in Europe²³ and Australia.²⁴ The recent NHANES (2005-2008) survey in the US found prevalence of 16.2%. However, the HSV-2 prevalence among non-Hispanic African Americans in that survey was comparable to our study, at 56%.²⁵ Similarly high prevalence (54%) has been noted in Haiti.

In our study, as in Haiti, Africa and Europe^{16,26,19}, we found that women aged 20 and above had higher prevalent HSV-2 infection. This high prevalence in women is similar to studies in Uganda where prevalence was about 50%²¹ in the national survey, 86% and 62% in a clinic sample of HIV-positive and HIV-negative pregnant women respectively⁹. Gender specific differences in HSV-2 infection have also been seen in Kenya and the Gambia^{22,27,28}. Consistent data from many populations in sub Saharan Africa shows higher prevalence of

HIV infection in women compared to men in population samples^{29,30,31}. Multivariable risk factors for prevalent HSV-2 infection (women, older age, low condom use, younger age at first sex and presence of HIV infection) were similar to earlier studies.¹⁸ Co-infection with HSV-2 and HIV has been documented in several studies^{22,34}. Kirakoya in addition to these risk factors, found an association between hormonal contraceptive use, bacterial vaginosis (BV) and incident HSV-2 infection³⁵ in women.

The gender differences for HSV-2 and HIV may be explained in part by the biological susceptibility of the female genital tract which makes women vulnerable to sexually transmitted diseases. Women may be vulnerable to HIV and HSV-2 due to innate biological factors. The highly prevalent herpes among women may contribute too³², as does the more efficient transmission of HIV from men to women. Probable increment in susceptibility of genital mucosa in the presence of hormonal contraceptives in women due to reduction in normal bacteria in the tract has been proposed as an explanation for the association between HSV-2 infection and contraceptive use³³ though hormonal contraceptive use may be a proxy for unprotected sexual intercourse. The documented synergy of HIV-1 and HSV-2 infection; and of contraceptives and HSV-2 infection in women in Africa, will need further exploration. Regarding HSV-2/HIV co-infection, recent studies have shown that treatment with acyclovir for HSV-2 did not have an impact on reduction of HIV transmission.^{6,36} A study by Zhu and colleagues showed that persistence and enrichment of HIV receptor cells (CD-4 T-cells) may create a good environment for the entry, attachment and dissemination of HIV in persons who have HSV-2 infection with or without ulceration. HSV-2 remains important for HIV transmission.

Socioeconomic factors may contribute to the gender disparity in prevalence of HSV-2. Education level, usually lower in women in developing nations may be a co-factor to explain the higher prevalence in women³⁸ as number of years in school is associated with safer sexual practices.

The higher prevalence in women too, may be enhanced by disparities in access to sexual health care of women in low income populations where cultural and social factors have been documented to influence access to care. Polygamy, increased number of sexual partners

especially among men, older age of partners coupled with early sexual debut by girls as shown in this study may further contribute to the higher risk for HSV-2 and resultant prevalence in women.

There was a similarity in HSV-2 prevalence in the 15-19 year categories for men and women, implying that this gender disparity in prevalence of HSV-2 only emerges in the female age groups with time. The study found lower HSV-2 prevalence younger age groups, notably similar in boys and girls. The lower, similar prevalence in boys and girls 15-19 years of age may present a window of opportunity to prevent herpes infection onset in an age cohort in this population. Targeting younger boys and girls for prevention of herpes could prevent new infections and enable potential control³⁹ and reduce the upward trend of HSV-2 prevalence. The nearly universal HSV-2 infection in the above 40 age group and the reported older of partner for young girls in this study, a phenomenon earlier documented in sub Saharan Africa⁴⁰, makes transmission likely to younger girls from older men. In turn, studies show younger girls also make sexual partnerships with younger boys^{40,41} with resultant transmission into their peer group.

Low prevalence of syphilis in recent years has been documented elsewhere in Africa⁴² and may be a result of the intensive campaigns for treatment of bacterial STDs. We found little self-awareness of genital herpes and an overwhelmingly inaccurate perception of etiology of genital ulcer disease in this population. Education on the high prevalence of HSV-2 as opposed to syphilis and HSV-2's atypical presentation for health workers and the population will be important to address this schism. Screening for HSV-2 is the best option but serological tests for genital herpes are still expensive. Community education may be of most benefit. An earlier paper published with colleagues, comparing different cut-offs for the HerpeSelect2, suggests the use of a 2.2 cut off¹². However, this report uses the higher 3.5 cut off. The higher cut-off increases specificity for the HerpeSelect 2 ELISA.^{14,43,44}

Limitations

Our study was limited in that distribution was skewed to women, despite return visits to increase men participation. We report a closely similar overall prevalence for the weighted analysis (weighted on 2002 Uganda census population distribution) for men and women. Another study limitation was the absence of a confirmatory

HSV-2 Western blot. However, the higher cut off point used increases the specificity of the test.

Conclusion

We reported high prevalence of HSV-2 and of HIV infection, especially among women in urban Kampala. HSV-2 infection was associated with being a woman, low condom use, early sexual debut. Prevalence increased with age. Combined with a lack of self awareness among those that they may be HSV-2 infected and with the high HIV prevalence, probability of HSV-2 transmission is high yet tests for herpes are limited to research settings in this community.

Health education on HSV-2 for health workers and the public is needed. A window of opportunity for prevention exists among the younger age groups. Behavioral interventions, especially in young women to delay sexual debut, reduce number of lifetime sexual partners and to increase consistent correct condom use could reduce HSV-2 incidence. The synergistic relationship between HSV-2, HIV and needs further exploration. The reasons underlying the divergence in gender specific prevalence with age, including contraception use also need further study. Cheaper, rapid tests for herpes simplex type 2 are needed. Since unaware, untested HSV-2 infected persons may be mixing freely within the population, the genital herpes epidemic may continue to grow in the absence of strong public health measures. Targeted public health engagement regarding HSV-2 in this population is crucial.

Acknowledgment

This work was supported by the National Institutes of Health, Fogarty International Center and the National Institutes of Health Office of Women's Health of the United States under the Global Research Initiative Program for New Foreign Investigators (GRIP) Grant Number TW006672-01 and the Government of Uganda, Ministry of Health.

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