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Original Article

Incidence and risk factors for herpes simplex virus type 2 seroconversion among pregnant women in Uganda: A prospective study

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Abstract

Introduction: Herpes simplex virus type 2 (HSV-2) acquired during pregnancy is associated with adverse outcomes such as perinatal HSV-2 transmission. HSV-2 seroconversion occurs within four weeks of HSV-2 acquisition. There was neither documented incidence nor risk factors for HSV-2 seroconversion during pregnancy in Uganda. The objective of this study was to determine the incidence and risk factors for HSV-2 seroconversion among pregnant women in Mulago Hospital, Uganda.

Methodology: A prospective study of 200 consenting HSV-2-negative women between 26 and 28 weeks of gestation was done between November 2013 and October 2014. HSV-2 serostatus was determined using HerpeSelect HSV-2 enzyme-linked immunosorbent assay (ELISA). Interviewer-administered questionnaires were used to collect socio-demographic characteristics and sexual history. Human immunodeficiency virus (HIV) serostatus was obtained from antenatal records. A total of 191 women completed follow-up and repeat HSV-2 serology by 38 weeks. Negative binomial regression analysis was used to estimate risk ratios for risk factors for HSV-2 seroconversion.

Results: Of 191 women, 15 (7.9%) seroconverted during pregnancy. Having multiple sexual partners, being in polygamous unions, and having HIV-positive serostatus were found to be risk factors for HSV-2 seroconversion.

Conclusions: The incidence of HSV-2 seroconversion during pregnancy in Uganda was high. Multiple sexual partners, polygamy, and HIV-positive serostatus were risk factors for HSV-2 seroconversion during pregnancy. Strengthening health education on the avoidance of multiple sexual partners during pregnancy is paramount in prevention of HSV-2 seroconversion.

Key words: herpes simplex virus type 2; incidence; seroconversion; pregnancy; risk factors.

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Introduction

Genital herpes simplex type 2 (HSV-2) is a sexually transmitted DNA virus and is prevalent in many geographical areas, especially among women 15–49 years of age [1]. The HSV-2 seroprevalence among women of childbearing age in developed countries ranges from 10.0%–40.0% compared to 30%–80% in developing countries [2-4]. The prevalence of HSV-2 among pregnant women in Zimbabwe was 49.1% [4, 5]. In Uganda, the HSV-2 seroprevalence among women in the general population was 49.0%. The HSV-2 prevalence was 67.2% among pregnant women in Uganda, and was even higher (86%) among pregnant women who were human immunodeficiency virus (HIV) seropositive [6].

The magnitude of HSV-2 acquisition among pregnant women is variable in the literature [5, 7]. Women develop antibodies to HSV-2 within 28 days of acquiring the primary infection [1]. HSV-2 seroconversion is an indicator of HSV-2 acquisition, especially if a previous test was negative [8]. The incidence of HSV-2 seroconversion during pregnancy was 2.0% in the United States of America [9]. In a Swiss cohort of pregnant women in Lausanne, there was no seroconversion detected during pregnancy [10]. In Italy, the incidence of HSV-2 acquisition in pregnancy was 3.0% [1]. In Zimbabwe, the seroconversion incidence was 4.2% (7/167) between the last four weeks of pregnancy and four months after childbirth [5].

The risk factors for HSV-2 acquisition are all related to sexual activity [1, 11]. Having multiple sexual partners or sex with a person who has multiple partners and young age of sexual debut were significantly

associated with HSV-2 acquisition [1, 5]. In addition, having any sexually transmitted diseases was a risk factor for HSV-2 seroconversion [8,12]. Condom use was found to be protective, especially if used correctly and consistently [13]. The specific factors that increase risk of HSV-2 seroconversion among pregnant women in Uganda had not previously been documented.

HSV-2 seroconversion during pregnancy has complications that are more common if the seroconversion occurs in the third trimester. These include HIV acquisition, perinatal transmission of HSV-2, plus preterm labor and delivery [1,14]. The risk of neonatal HSV-2 acquisition is high in new HSV-2 infections acquired during pregnancy [3]. Seventy to ninety percent of infants with neonatal HSV-2 are born to women who seroconvert to HSV-2 during pregnancy [3,15]. In Uganda, the neonatal mortality rate is still high, at 26 per 1,000 live births [16]. Prematurity contributed about 24.0% of all neonatal deaths in one study in Uganda [17]. The burden of neonatal herpes infection and the contribution of genital herpes to premature delivery in Uganda is still unknown, although in neighboring Kenya, the incidence of neonatal herpes was estimated to be 33 per 100,000 live births [18]. Knowing the incidence of HSV-2 seroconversion is a start to ascertain whether it has a significance that would warrant further studies to determine the burden of undesirable obstetric outcomes such as neonatal herpes among women who seroconvert during pregnancy. These studies would then be followed by an increase of the interventions aimed at reducing adverse obstetric outcomes due to of HSV-2 infection during pregnancy.

Studies on the role of HIV infection in HSV-2 infection have yielded conflicting results. HIV-positive women were more likely to be HSV-2 positive [14]. In Cameroon, the sero-prevalence of HSV-2 in HIV-infected women was 70.0% [19]. HIV-positive women were five times more likely to have HSV-2 infection than were HIV-negative women in Mulago Hospital. However, in a study by Munjoma *et al.* (2009), HIV serostatus was not found to be a risk factor for HSV-2 seroconversion in pregnancy [5]. There is a need to further explore a possible association between HIV and HSV-2 seroconversion.

In Uganda, with a high HSV-2 prevalence of 49.0% among women, 67.2% among pregnant women, and 86.0% among HIV-positive pregnant women, there was scarce data regarding incidence of HSV-2 seroconversion during pregnancy. In addition, the specific risk factors for HSV-2 seroconversion during pregnancy were not known. This study aimed to determine the incidence and risk factors for HSV-2 seroconversion among pregnant women in Mulago Hospital, Uganda.

Methodology

Study design, site, and population

A prospective study was conducted in the antenatal clinic, antenatal ward, and labor ward of Mulago Hospital from November 2013 to October 2014. In Mulago Hospital, all women are routinely screened for HIV, and those who test positive are given antiretroviral therapy [6]. From the hospital records, 50% of the women seen in the hospital are unemployed, about 90% are married or cohabiting, and two-thirds are Christians. Women between 26 and 28 weeks of pregnancy and those who were HSV-2 negative, 18 years of age and older, and had consented to the study were enrolled. The purpose of screening women between 26 and 28 weeks was to allow sufficient time to have test results by the onset of the third trimester. Assuming an incidence of HSV-2 seroconversion of (12% after nine-month follow-up) [5], using the formula for single cohort without a comparison group in which the outcome was proportion, a level of precision of 5% and power of 80%, with confidence level of 95%, the estimated minimum sample size was 168. Adjusting for 20% loss to follow-up, the sample size was 200 participants.

The primary outcome variable was HSV-2 serological status (positive or negative) determined by HerpeSelect HSV-2 enzyme-linked immunosorbent assay (ELISA) (Focus Diagnostics, Cypress, USA) by 38 weeks. The independent variables were socio-demographic characteristics (age, marital status, level of education, and occupation), obstetric history (parity, history of preterm labour and premature rupture of membranes), and sexual history (age of sexual debut, number of sexual partners since sexual debut, number of sexual partners in the last six months, condom use, and number of wives the partner has). Additional independent variables were HIV serostatus obtained from the participants' antenatal records and presence of concurrent genital *Trichomonas vaginalis* and candidiasis.

Ethics statement

Ethical approval was obtained from the Makerere University College of Health Sciences School of Medicine Research and Ethics Committee, and the Uganda National Council of Science and Technology (approval number HS 1413). Written informed consent was obtained from all the participants who were screened and enrolled. All participants had received

health education about HSV-2 prevention. The participants who seroconverted to HSV-2 were given acyclovir to prevent their babies from acquiring HSV-2 perinatally. The data collected included sensitive information such as sexual practices; thus, confidentiality was observed for all the study documentation, and records were placed under double lock and key.

Data collection

Consenting women had 2 mL of blood drawn from the cubital vein into a plain vacutainer (Becton, Dickinson and Company, Franklin Lakes, USA). The blood sample was taken to the immunology laboratory of the Makerere University College of Health Sciences. Serum was recovered the same day from the blood samples and was tested for HSV-2 IgG antibodies using the HerpeSelect HSV-2 ELISA IgG to glycoprotein G (Focus Diagnostics, Cypress, USA), following the manufacturer's instructions [20]. The test has a sensitivity of 96%–97% and a specificity of 98% [20]. The last stage of the test procedure involves adding a reagent that forms color when HSV-2 antibodies and antigens react. The ensuing color change is measured by a spectrophotometric reading of optical density (OD). Sample optical density readings are recorded and compared with reference cut-off OD readings to determine results. The results are reported as positive or negative. At screening for HSV-2 negative women for enrollment, the manufacturer's directions were followed, where index values < 0.9 were negative and 1.1 and above were positive. Index values between 0.9 and 1.0 (inclusive) were equivocal. The equivocal results were repeated. When the cut-off of index values < 0.9 were taken as negative, a review by Biraro *et al.* (2011) reported that the sensitivity of the test was over 99% [21]. For detection of seroconversion, taking the cut-off of 1.1 and above as positive had low specificity (less than 70%) [21]. The cut-off optical density of 3.5 and above was therefore considered to be positive for seroconversion and below that to be negative, similar to what has been the recent practice in African studies [20, 22].

Socio-demographic data were obtained using a case report form. The HIV serostatus obtained from the antenatal records is routinely performed in Uganda using rapid lateral flow systems according to a serial algorithm that begins with the HIV Determine Kit (Abbott Laboratories, Chicago, USA). All positive samples were confirmed with STATPAK dipstick strips (Chembio Diagnostic Systems, Inc., New York, USA) and discrepant results confirmed with Uni-Gold (Trinity Biotech Plc., Bray, Ireland). Two high vaginal swabs were also taken at the enrollment stage; one swab was for culture and sensitivity for yeast cells and the second swab for DNA polymerase chain reaction (PCR) for *Trichomonas vaginalis* [23, 24]. The participants were followed up monthly for routine antenatal check-ups. Any illnesses were appropriately treated, and routine supplements such as iron, folic acid, and calcium were given. The HerpeSelect HSV-2 ELISA was repeated at term (38 weeks) or earlier if a participant went into labor, in which case the test was repeated at the time of admission into the labor ward.

The research assistants who were doctors and midwives were trained in counseling patients about the research, obtaining consent, and collecting data and specimens. Data was cleaned daily by the principal investigator prior to double data entry into the Epi Info 7 program (CDC, Georgia, USA). It was then exported to STATA version 12.1 statistical program (Stata Corp., College Station, USA) for analysis.

Data analysis

Categorical data were summarized as counts and proportions while continuous variables such as age were summarized using means and standard deviations. Some continuous variables such as age of sexual debut were re-categorized into meaningful strata to be used for subsequent analysis. The incidence of HSV-2 seroconversion in the third trimester was calculated as the number of those who seroconverted by 38 weeks divided by the total that completed follow-up, and was expressed as a percentage. Negative binomial regression analysis was used for bivariate and multivariable analysis to calculate risk ratios for risk factors for HSV-2 acquisition. The reason for using this type of analysis model was because the outcome variable was whether seroconversion occurred or not [25]. Variables that had a p value of less than 0.1 at bivariate analysis and those that had a biologically plausible link to HSV-2 were included in the multivariable model to determine the factors independently associated with seroconversion. The loss to follow-up was 4.5%. A sensitivity analysis in which all those lost to follow-up were considered positive and another in which they were considered negative led to similar results.

Results

Two hundred participants were enrolled for the study. A total of 524 women were screened for HSV-2 serology. Of the women screened, 247 were HSV-2 negative, of which 47 were excluded because they

missed their enrollment appointments. The HSV-2 seroprevalence rate among the women in Mulago Hospital antenatal clinic at the time of screening was 52.9% (277/524). The mean age of the participants was 25.5 ± 5.0 years. A total of 191 participants had a repeat HSV-2 serology done either at delivery if before 38 weeks or else at 38 weeks. The 9 women who were lost to follow-up never came for the closing study visit and also delivered elsewhere; thus, no repeat HSV-2 serology was performed.

Of the 200 women enrolled, 191 (95.5%) successfully completed the 10 weeks of follow-up and a closing HSV-2 serology was done. Of the 200 women enrolled, 69.0% were 20–29 years of age and 82.0% were multiparous; 76% had attained at least secondary education, but only 50.0% of them were employed. While the majority of the participants (93.5%) were married, only 30.5% reported condom use, and 5.0% reported having more than one sexual partner in the past six months.

A total of 191 pregnant women completed follow-up, and 15 of them seroconverted by 38 weeks of gestation, giving an incidence of 7.9%. Seventeen participants had preterm delivery; three of them seroconverted to HSV-2. Only one of the women who seroconverted (6.7%) had symptoms (vulval and vaginal vesicles). She was given a treatment course of acyclovir. Table 1 compares the socio-demographic characteristics of the women who seroconverted to HSV-2 compared to those who did not seroconvert. The women who seroconverted to HSV-2 had similar age, education, marital status, occupation, and gravidity compared to those who did not seroconvert.

Factors associated with seroconversion

Table 2 shows both bivariate and multivariable analysis for risk factors for HSV-2 seroconversion. Participants who had their sexual debut at 16 years of age or older had reduced risk of seroconversion for HSV-2 seroconversion (RR: 0.28; 95% CI: 0.11–0.77). Women who had multiple sexual partners in the preceding six months and women in polygamous marriages were at increased risk of HSV-2 seroconversion (RR: 4.53; 95% CI: 1.51–13.50) and (RR: 6.31; 95% CI: 2.09–19.0), respectively. In contrast, there was no association between condom use and HSV-2 seroconversion (RR: 1.6; 95% CI: 0.54–3.90).

The association of HSV-2 seroconversion with HIV serostatus, candidiasis, and *Trichomonas vaginalis* was tested. HSV-2 seroconversion was associated HIV-positive status (RR: 5.72; 95% CI: 2.00–16.32) but was not associated with *Trichomonas vaginalis* (RR: 3.11; 95% CI, 0.82–11.76) or candidiasis (RR: 1.42; 95% CI: 0.53–3.84).

In order to assess for interaction, the relationship between HIV serostatus and the following variables was assessed: age of the participants, condom use, multiple sexual partners in the preceding six months, and presence of *Trichomonas vaginalis* infection. No association of HIV was found with any of them.

In the final adjusted model, multiple sexual partners in the preceding six months and being in polygamous

Table 1. Characteristics by herpes simplex virus 2 (HSV-2) seroconversion status among pregnant women in Mulago Hospital, Uganda, November 2013 to October 2014.

Characteristics/categories	Seroconverted N = 15	Not Seroconverted N = 176
Mean age in years ± SD	25.6 ± 5.3	25.7 ± 4.9
	n (%)	**n (%)**
Education		
Less or equal to primary	4 (26.7)	43 (24.4)
Secondary and above	11 (73.3)	133 (75.6)
Marital status		
Not married	2 (13.3)	10 (5.7)
Married/cohabiting	13 (86.7)	166 (94.3)
Occupation		
Business	4 (26.7)	56 (31.8)
Formal employment	2 (13.3)	37 (21.0)
Unemployed	9 (60)	83 (47.2)
Gravidity		
Prime gravida	5 (33.3)	34 (19.3)
Multipara (2–4)	6 (40.0)	113 (64.2)
Grand multipara	4 (26.7)	29 (16.5)

Table 2. Risk factors for herpes simplex virus 2 (HSV-2) seroconversion by 38 weeks in Mulago Hospital, Uganda, November 2013 to October 2014.

Characteristics /categories	Total N	Seroconversion n (%)	Risk ratios[i] (RR) (95% CI)	P values	Adjusted risk[ii] ratios (aRR)	P values
Age of sexual debut (yrs)						
Less than 16	24	5 (20.8)	1.0			
16 and above	167	10 (6.0)	0.28 (0.11-0.77)	0.013	0.33 (0.21–0.48)	< 0.001
Partners since sex debut						
1	115	3 (4.0)	1.0			
2 or more	76	12 (10.5)	2.64 (0.77–9.06)	0.122	-	-
Partners in last 6 months						
1	181	12 (6.6)	1.0			
2 or more	10	3 (30.0)	4.53 (1.51–13.50)	0.007	3.22 (2.01–5.18)	< 0.001
In polygamous union						
No	133	4 (3.0)	1.0			
Yes	58	11 (19.0)	6.31 (2.09–19.0)	0.001	4.24 (1.37–13.26)	0.012
Used condoms						
No	131	9 (6.9)	1.0			
Yes	60	6 (10.0)	1.46 (0.54–3.90)	0.456	1.40 (0.63–3.11)	0.408
HIV status						
Negative	183	12 (6.6)	1.0			
Positive	8	3 (37.5)	5.72 (2.00–16.32)	0.001	4.35 (2.15–8.81)	< 0.001
Trichomonas vaginalis						
No	182	13 (7.1)	1.0			
Yes	9	2 (22.2)	3.11 (0.82–11.76)	0.094	2.09 (0.52–8.38)	0.296
Candidiasis						
No	93	6 (6.5)	1.0		-	-
Yes	98	9 (9.2)	1.42 (0.53–3.84)	0.486		
TOTAL	**191**	**15 (7.9)**				

[i] Negative binomial regression analysis was used; [ii] The risk ratios are adjusted for all other significant independent variables in the final model.

unions were risk factors for HSV-2 seroconversion (aRR: 3.22; 95% CI: 2.01–5.18) and (aRR: 4.24; 95% CI: 1.37–13.26), respectively. In contrast, delaying sexual debut to 16 years of age or older reduced the risk of HSV-2 seroconversion (aRR: 0.33; 95% CI:0.21–0.48).

Discussion

In this study, we set out to determine the incidence and risk factors for HSV-2 seroconversion among pregnant women in Mulago Hospital, Uganda. The incidence of HSV-2 seroconversion was 7.9%. Having multiple sexual partners increased the risk of HSV-2 seroconversion threefold compared to having one partner. Having a polygamous partner increased the risk of HSV-2 seroconversion fourfold compared with women whose spouse had only one partner. Being HIV positive increased the risk of HSV-2 seroconversion fourfold compared to being HIV negative.

The incidence of HSV-2 seroconversion during pregnancy in our study was higher than that reported from the United States of America, Switzerland, and Italy [1, 7, 10]. This incidence was also higher than that reported by Munjoma *et al.* (2010) in Zimbabwe between late pregnancy and four months after delivery [5]. After a longer period of follow-up, the incidence of HSV-2 seroconversion increases, as evidenced by the Zimbabwe study where, after a follow-up period up to nine months after delivery, the incidence increased from 4.2% to 12% [5]. The higher incidence of HSV-2 over a shorter follow-up in Uganda may be explained by the fact that the population level of HSV-2 is generally high, leading to higher rates of contact between infected and uninfected persons [6, 22, 26]. The seroprevalence of HSV-2 among women in Kampala was above 60% [6, 22]. However, in Italy, where the HSV-2 seroprevalence among pregnant women was only an eighth of the seroprevalence in Uganda, the incidence of HSV-2 seroconversion during pregnancy was also low (3%) [1]. In a Zimbabwean cohort of pregnant women screened from a population with HSV-2 seroprevalence of 49.1%, the incidence of HSV-2 seroconversion was 4.2%.

In our study sample population, 5% of the women had multiple sexual partners during pregnancy. Having multiple sexual partners and being in polygamous unions increased the risk of HSV-2 acquisition threefold and fourfold, respectively. Other studies also

found multiple sexual partners and polygamy to be risk factors for HSV-2 seroconversion [1]. Having multiple sexual partners increases the risk of one of the partners having HSV-2 and transmitting to the others.

Young age of sexual debut (under 16 years) was a risk factor for HSV-2 seroconversion during pregnancy in our study. In Zimbabwe, where young age of sexual debut was also identified a risk factor for HSV-2 acquisition, the risk was related to cross-generation sex between young women and older men [5]. However, we did not collect data on the age of partners at sexual debut of our participants.

Condom use was not protective against HSV-2 acquisition in our study. This could be due to the low condom use in our study (30.5%). Women only used condoms in what they perceived to be risky sex. Condom use is known to be protective against genital infections, including HSV-2 [13]. We did not, however, assess if condoms were correctly or consistently used.

HIV serostatus was a risk factor for HSV-2 seroconversion in our study. The association between HIV and HSV-2 has been documented in previous studies [6, 27, 28]. It is known that since patients with acquired immunodeficiency syndrome (AIDS) are more likely to have other genital infections, there would be a higher risk of HSV-2 acquisition in HIV-positive pregnant women compared to HIV-negative pregnant women [29]. New HSV-2 and genital ulcers increase acquisition of HIV infections via antigen presenting cells [27]. In addition, HIV-positive women who have HSV-2 are more likely to have genital secretions that contain herpes DNA than do HIV-negative women [30]. Furthermore, Corey *et al.*, (2004) in a review article, reported that HIV-positive non-pregnant women in rural Uganda had a threefold risk of HSV-2 seroconversion compared to their HIV-negative counterparts, although no conclusive explanation for this was given [31]. *Trichomonas vaginalis* and candidiasis, which were the co-genital infections tested in our study, were not associated with the risk of HSV-2 seroconversion. Similarly, *Trichomonas vaginalis* in a study by Munjoma *et al.* (2010) was also not a risk factor for HSV-2 seroconversion, although the women who were HSV-2 seropositive at baseline were more likely to have *Trichomonas vaginalis* [5]. The finding, therefore, that HIV-positive women in our study were more likely to seroconvert to HSV-2 than were HIV-negative women was probably due to shared risk of multiple sexual partners rather than the genital infections.

Limitations of our study include few (n = 8) HIV-positive patients from the sample of 191 analyzed; thus, further analysis for interactions was not possible. We also did not assess specifically whether condoms were used correctly or consistently during the study period. In addition, we did not have partner HSV-2 serostatus or partner socio-demographic characteristics to be able to explain the HSV-2 transmission factors extensively. The sample size was not adequate to assess interaction between the different risk factors for HSV-2 seroconversion. We also did not do confirmatory tests for HSV-2 serostatus using Western blot (developed at Washington University: UW-WB). However, we used a cut-off for HerpeSelect HSV-2 ELISA that has been known to approximate specificity of over 90% in studies done in Africa [20].

Conclusions

The incidence of HSV-2 seroconversion among pregnant women in Mulago Hospital, Uganda, was high. The risk factors for seroconversion were having multiple sexual partners, being in polygamous unions, being HIV positive, and sexual debut at under 16 years of age.

This magnitude of HSV-2 seroconversion implies that the complications of HSV-2 during pregnancy such as preterm labor and perinatal transmission of HSV-2 may be common as well. There is a need, therefore, to determine the prevalence of neonatal herpes in Uganda. In addition, there is a need to strengthen heath education for pregnant women on the prevention of genital HSV-2 by avoiding multiple sexual partners. Furthermore, at the booking visit, women at risk of HSV-2 seroconversion may need routine HSV-2 serological screening and a repeat test to be done in the third trimester for evidence of seroconversion. Those who seroconvert would then receive acyclovir, which is known to reduce transmission of HSV-2 to the neonates. We also need implementation studies to determine the uptake and effectiveness of routine suppressive acyclovir given from 36 weeks to HSV-2-positive pregnant women in developing countries with a high prevalence of HSV-2. Additionally, we need a larger study to examine the relationship between HIV serostatus and incident genital herpes infection so as to reduce both HIV and HSV-2 transmission.

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Authors' contribution

SN designed the study from conception, performed the data collection and statistical analysis, and wrote the manuscript. DKK, NMT, and FMM reviewed the study from the design stage, participated in statistical analysis, and contributed to writing the manuscript. FB and ENJ reviewed the design and participated in writing the manuscript.

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