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The Epidemiology of Herpes Simplex Virus Type-2 Infection Among Married Women in Mysore, India

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HERPES SIMPLEX VIRUS TYPE-2 (HSV-2) is one of the most common sexually transmitted diseases in the world.[1] Although the prevalence of HSV-2 infection varies widely by country and population subgroup,[2] generally higher rates are found in developing countries where the prevalence in adults can reach 50%.[3] Although data are sparse for India, studies have shown HSV-2 seroprevalences among sexually transmitted diseases clinic attendees[4,5] ranging from 43% to 83%, and lower prevalences in population-based cross-sectional studies,[6–8] from 7.9% to 14.6%.

There is growing evidence that HSV-2 infection, in addition to being a significant source of morbidity, is a risk factor for human immunodeficiency virus (HIV) acquisition and transmission.[9] One study showed a 3-fold increased risk for HIV infection among individuals with recent seroconversion to HSV-2.[4] This raises the possibility that HSV-2 infection may be a key determinant of HIV incidence rates in populations, and that HSV-2 detection and management of herpes infections may offer a prevention strategy for HIV.

Cross-sectional analyses were conducted of baseline findings of a prospective cohort study of young married women that examined the relationship of lower genital tract infection to acquisition of HSV-2 infection. Study participants were recruited between November 2005 and March 2006. Potential participants were approached in 2 ways: in the obstetrics gynecology outpatient clinic at CSI Holdsworth Memorial Hospital (HMH), and through outreach in the community where women were invited to the study site for screening. To be included in the study, participants were between 15 and 30 years of age, sexually active, post defined as having had vaginal intercourse at least once in the 3 months, willing to undergo pelvic examination, and planning to stay in the area for 6 months. The study was approved by the Ethical Review Board at the University of California, Berkeley, and Asha Kirana Hospital. Epidemiologic data were collected in Kannada from all eligible participants who gave written informed consent. After completion of the interview, participants underwent a clinical examination and laboratory evaluation for reproductive tract infections (RTI).

Diagnostic testing was completed at HMH and Vikram Hospital, Mysore. A type-specific ELISA test was used to detect HSV-2 antibodies (Focus Technologies, Cypress, CA). Manufacturer's instructions were followed using an index value of >1.1 as positive HSV-2 specimen. Gram stained vaginal smears were independently assessed for bacterial vaginosis (BV) with Nugent score[10] by 2 trained study personnel. Vaginal swabs were cultured for *Trichomonas vaginalis* (TV) (InPouch, Biomed Diagnostic, OR); and for *candida* species (BioMed Diagnostics, OR). Endocervical swabs were cultured for *Neisseria gonorrhoeae* (GC) (BioMed Diagnostics, OR). Validation of HSV testing was performed using a masked panel of serum specimens from the University of California, San Francisco. Results yielded 100% agreement. The first 300 sera were run in duplicate for reproducibility of test results. The GC culture system was validated for growth of GC with 3 ATCC strains (49226, 31426, and 19424). There was independent verification of 10% of tests for *T. vaginalis* and candida by an independent microbiologist, whose results matched the study findings.

The primary study outcome was seropositivity for HSV-2 antibodies, analyzed as a binomial variable. Univariate associations of baseline characteristics with HSV-2 seroprevalence were made using Pearson χ^2 test or Fisher exact method. Variables with P values <0.1 were considered for inclusion in a multivariable logistic regression model. Stata 9.0 (Stata Corporation, TX) was used for statistical analysis.

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Of the 2131 women screened, 1193 were eligible. Reasons for noneligibility included not living in the geographical area, age, not being sexually active in prior 3 months, pregnancy, and having vaginal bleeding. Of those, 996 (83%) agreed to participate and 898 (75%) completed all baseline procedures and were included in the study. The mean age of participants was 25.8 years (range: 16–30 years). Most (69%) women were Hindus and 29% were Muslims. Women's mean age at menarche was 13 years, and at marriage, 16.9 years. Almost half (49%) of participants had more than 5 years of education, but 26% had no schooling. Reversible methods of family planning were not common, with 4% using intrauterine devices, 2% oral contraceptives, and 5% condoms or abstinence. Tubectomy was common (63%) and 25% used no contraception. Most women (84.5%) reported having children. Seventy-four percent were housewives, 20% worked as unskilled laborers, and 6% had other occupations. Among their husbands, 45% worked as unskilled laborers, 43% performed skilled jobs, and another 11% were drivers. About 82% of study participants reported their total monthly household income as ≤4,000 INR (93 USD).

Eighteen women reported more than one sexual partner in their lifetime, and 4 of those reported new sexual partners in the prior three months. Ninety-four percent reported having never used condoms, whereas 2% reported consistent condom use. The median number of vaginal sex acts in the prior 3 months was 24 (range 2–120). Only 56 (6.1%) women reported ever having had anal sex, of whom 41 reported it in the prior 3 months. Of the study participants, 40% of women reported having sex while their partner was under the influence of alcohol, and 98% of those sexual acts were unprotected in the prior 3 months.

Overall, 53.9% of the 898 women had laboratory-diagnosed RTI: 9% had trichomoniasis (95% confidence interval, CI: 8.6%–10.5%); BV was diagnosed in 19% (CI: 16.6%–21.8%); and candidiasis was

TABLE 1. Correlates of HSV-2 Infections Among 882 Young Married Women in Mysore, India

Characteristic	Unadjusted OR	95% CI	aOR*	95% CI
Duration of sexual activity(per yr of sexual activity)†	1.05‡	1.00–1.11		
Age (yr)				
15–20	1.00		1.00	
21–25	1.60	0.47–5.43	2.26	0.66–7.83
26–30	2.96	0.90–9.74	3.59‡	1.07–12.01
Yrs. of education (yr)				
>7	1.00		1.00	
0–7	1.87‡	1.22–2.86	2.46§	1.57–3.83
Age at marriage				
≤18	1.05	0.64–1.69		
>18 yr	1.00			
Religion				
Muslim	0.22§	0.11–0.44	0.19§	0.09–0.38
Non-Muslim	1.00		1.00	
Occupation of the husband				
Skilled	1.00		1.00	
Skilled-drivers	1.57	0.77–3.17	1.86	0.89–3.86
Unskilled	1.89‡	1.19–3.00	1.63‡	1.01–2.62
Have children				
No	1.77‡	1.07–2.93	3.05§	1.71–5.45
Yes	1.00		1.00	
Past history of genital ulcer				
No	1.00		1.00	
Yes	2.07§	1.15–3.75	2.29§	1.24–4.26
Cigarette smoking by partner				
No	1.00			
Yes	1.41	0.93–2.14		
Alcohol use by partner				
No	1.00			
Yes	1.84§	1.21–2.80		
Sex under the influence of alcohol (last 3 mo)				
No	1.00			
Yes	1.57‡	1.04–2.39		
Bacterial vaginosis (Nugent)				
Negative (0–3)	1.00		1.00	
Intermediate (4–6)	0.94	0.49–1.81	0.88	0.45–1.72
Positive (7–10)	2.18§	1.35–3.54	1.82‡	1.1–3.00
Trichomoniasis				
Negative	1.00		1.00	
Positive	3.89§	2.24–6.72	3.17§	1.78–5.65

*OR adjusted for age, religion, and education.
†Duration of sexual activity and age were correlated, hence duration of sexual activity was not included in the multivariable model.
‡$P < 0.05$.
§$P < 0.005$.

detected in 30% (CI: 27.9%–33.9%). Gonorrhea was not detected. Of the women whose blood was available for serologic testing (n = 882), 100 (11.3%) were HSV-2 seropositive (CI: 9.4%–13.6%). HSV-2 prevalence increased with age: 5.4% among women aged 15–20 years; 8.3% among 21–25 years; and 14.4% in women over 25 years of age (P value = 0.02). Among those with HSV-2 antibodies, only one had genital ulcers on examination. Of the women who had trichomoniasis, 29% were HSV-2 positive, when compared with 9% of women who were not diagnosed with trichomoniasis (P value <0.001). Similarly 19% of women who had BV had HSV-2 infection when compared with only 10% of women who were negative for BV (P value = 0.003). Twenty-two percent of women who gave a history of genital ulcers or sores had HSV-2 antibodies when compared with 10% reporting no genital ulcers (P value = 0.001). Fourteen percent of women reporting sex with a partner under the influence of alcohol were HSV-2 positive, compared with 9% of women who had sex with a partner not under the influence of alcohol (P value = 0.033). Fifteen percent of women with partners who used alcohol had HSV-2 antibodies when compared with 8% of women who reported partners not using alcohol (P value = 0.003). Similarly, 13% of women with partners who smoked had HSV-2 antibodies when compared with 9% of women who reported their partners were nonsmokers (P value = 0.09).

Univariate analysis showed relative odds of having HSV-2 antibodies increased with age, less education, being non-Muslim, not having children, history of genital ulcers, sex while a partner was under the influence of alcohol, partner's occupation, partner's smoking and alcohol use, and presence of a detectable RTI such as trichomoniasis or BV (Table 1). Multivariable logistic regression showed increasing age, less education, being non-Muslim, having an unskilled laborer as a partner, not having children, history of genital ulcers, and having detectable trichomoniasis or BV were independently associated with HSV-2 seropositivity in the adjusted model (Table 1).

This study corroborates findings of research elsewhere in India. With an HSV-2 seroprevalence of 11.3%, study results fall within the range (7.9%–14.6%) found in other community-based populations.[6–8] Consistent with other research,[11,12] an association between BV and HSV-2 infection was found, although it disappeared on adjustment for trichomoniasis. It is possible that these findings are related to residual confounding with sexual behaviors, or are the result of unmeasured interaction between BV and trichomoniasis. An association between trichomoniasis and HSV-2 infection has been described in previous studies.[13–15] Because of the cross-sectional nature of our analysis, it is impossible to establish temporality among these infections to better understand this relationship. In our study, Muslim women were less likely to be seropositive for HSV-2 antibodies. It is possible that there are cultural factors that reduce the risk of infection among Muslim women. Additional research is needed to understand these sociocultural factors.

Our study had several limitations. There could be information bias as sexual histories were self-reported and retrospective. It is possible that women underreported sexual partners owing to social-desirability bias. Because participants were not knowledgeable about their partners, it is likely that there is misclassification regarding partner's alcohol and drug use. Because the study relied on a convenience sample, findings may not be generalizable to other populations. It is possible that the study overestimated the prevalence of HSV-2 infection. Currently, there is a scientific debate about the accuracy of Focus ELISA test in areas of low prevalence.[16–19] Some researchers have recommended raising the cutoff value to ≥3.5 from the manufacturer recommended cutoff of >1.1 to reduce false-positive results in areas of low prevalence.[17]

In conclusion, our study found low condom use and a high burden of laboratory-diagnosed RTI among young married women in this population. These findings suggest an unmet need for effective RTI care for young married women. In addition, further research is needed to understand the relationship among BV, trichomoniasis, and HSV-2 infection.

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