Incidence of herpes simplex virus type 2 in young reproductive age women in Mysore, India

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Abstract

Context—There are sparse data on herpes simplex virus type 2 (HSV-2) infection in India. HSV-2 is one of the most common sexually transmitted infections and the primary cause of genital ulcer disease worldwide.

Aim—The aim of this study is to describe the incidence of HSV-2 infection among young reproductive age women in Mysore, India.

Setting and Design—Between October 2005 and April 2006, 898 women were enrolled into a prospective cohort study in Mysore, India, and followed quarterly for 6 months.

Materials and Methods—An interviewer administered questionnaire was used to collect demographic and social risk factors, and physical examination was conducted for collection of biological specimens to screen for reproductive tract infections at each visit. Serologic testing was conducted for the presence of HSV-2 antibodies using HerpeSelect HSV-2 enzyme-linked immunosorbent assay.

Statistical Analysis Used—Data were analyzed using R. Incidence density rates were calculated using Poisson distributions with person-time of follow-up as denominator. Person-time was calculated as time from enrollment until time of first positive HSV-2 test.

Results—There were 107 women with HSV-2 antibodies leaving 700 women with negative results at enrollment. The analysis included 696 out of which, there were 36 HSV-2 seroconversions during the study period. The study cohort accumulated roughly 348 woman-years of follow-up, yielding an HSV-2 acquisition rate of 10.4 cases/100 woman-years. All detected infections were asymptomatic.

Conclusions—HSV-2 incidence is moderate in this community sample of young reproductive age monogamous women. More research is needed to establish incidence estimates in different Indian settings.

Keywords
Herpes simplex virus; herpes simplex virus type 2; incidence; India; Women
INTRODUCTION

Herpes simplex virus type 2 (HSV-2) is one of the most common sexually transmitted infections (STI), and the primary cause of genital ulcer disease worldwide.\[1\] According to WHO, approximately 60% of worldwide HSV-2 infections are among women. For 2003, it was estimated that there were 23.6 million new HSV-2 infections among 15- to 49-year olds globally, with 12.8 million of those infections among reproductive age females.\[2\]

There is an acute lack of data on HSV-2 incidence in India. There are only two studies among women attending sexually transmitted disease clinics and HIV voluntary counseling and testing centers in India that showed an incidence of 8% and 11.4 cases/100 person-years in Chennai and Pune, respectively.\[3,4\] This article describes the incidence of HSV-2 infection in a cohort of reproductive age women in Mysore, India.

MATERIALS AND METHODS

Women were recruited into a prospective cohort study to examine the relationship of vaginal infections and HSV-2 acquisition using recruitment procedures described elsewhere.\[5\] In brief, the participants were eligible to participate if they were between 15 and 30 years of age, sexually active, willing to undergo a pelvic examination, and able to give informed consent. The study was approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley, and the Asha Kirana Institutional Review Board in compliance with all regulations governing the protection of human subjects. All women provided written informed consent before participating in the study.

Between October 2005 and April 2006, 906 women were enrolled. A total of 898 women completed all the baseline procedures and were included in the study to be followed-up for 6 months. At the baseline visit and subsequent two quarterly visits, the participating women were interviewed in the local language of Kannada about demographics, risk behaviors, and reproductive health using a structured questionnaire. All the participants also underwent a pelvic examination and specimens were collected to test for reproductive tract infections. A serum sample was obtained from each participant to test for HSV-2 antibodies. Serum was frozen and stored at −70°C until HSV-2 antibody testing could be completed.

All serologies were conducted at the Vikram Hospital laboratory. The serum samples were tested for type-specific antibodies to HSV-2, using a commercially available enzyme-linked immunosorbent assay (ELISA; Focus Technologies, Cypress, CA) according to the manufacturer’s instructions. An index value > 1.10 was considered to be positive, and values < 0.90 were considered to be negative. Serum samples for which the results were equivocal (an index value of 0.90–1.10) were retested. The results for a particular specimen were considered equivocal if the second index value was between 0.90 and 1.10. Acquisition of HSV-2 was defined as a change in the ELISA index value from <0.9 (negative) at enrollment to >1.1 (positive) at the 3rd or 6th month follow-up visit. Women who tested HSV-2 negative at enrollment and positive at 6 months, stored sera from the 3rd month visit was tested to determine the timing of HSV-2 seroconversion.

For validation of the HSV testing methodology, University of California San Francisco sent a panel of unknown sera specimens to India. The panel consisted of 10 positive and 10 negative specimens. The samples were tested blind and the results yielded 100% agreement. In addition, the first 300 sera were run in duplicate for reproducibility of test results. Each ELISA assay was reviewed for abnormal trends and patterns of testing. All positives and a random sample of negative sera were retested at a reference laboratory in India yielding concordant results.
Data were analyzed using R (R Foundation for Statistical Computing, Vienna, Australia). Incidence density rates were calculated using Poisson distributions with person-time of follow-up as the denominator. Person-time was calculated as time from enrollment until time of first positive HSV-2 test. We selected a short list of demographic and clinical variables of interest a priori based on the literature. The values for these variables were generally available for several time points; we chose to use only the baseline covariate values for the present analysis. Continuous variables were recoded as categorical variables based on select groupings that were common or likely to be intuitive.

Missing values for demographic or clinical variables were excluded from the analysis. Missing HSV-2 results were imputed via the assumption that HSV-2 infection is persistent (individuals negative at follow-up were assumed to be negative at baseline and individuals positive at baseline were assumed to be positive at follow-up). Analyses were restricted to women who were negative at baseline and who completed the survey at baseline. This resulted in a cohort of 689 women. Descriptive analyses were conducted using Fisher’s exact test for categorical variables. A significance level of alpha at 0.05 was assumed for all tests.

RESULTS

The study enrolled 898 women, of whom blood samples were available from 882 women for serologic testing. Of those, 807 women returned for at least 1 follow-up visit. The retention rate was 94.8% at 3 months and 91% at 6 months.

Based on the standard definition, 107 women were positive for HSV-2 antibodies leaving 700 women with negative results at enrollment. The analysis included 696 women as three had no survey data and one woman had incomplete serology history. There were 36 HSV-2 seroconversions during the study period. The study cohort accumulated roughly 348 woman-years of follow-up, yielding an HSV-2 acquisition rate of 10.4 cases/100 woman-years.

The characteristics of the study population are described in Table 1. Using the manufacturer’s suggested index cutoff value to define seropositivity, 696 women were included in this analysis, 473 (69.3%) described themselves as Hindus, 210 (30.7%) as Muslims, and 13 (1.9%) as Christians. Almost all the women were married and reported being monogamous. A majority of the participants in this study were >26 years of age (52.6%) and had less than 7 years of education (54%). Most of them had children (86.6%), and 64% of the women had undergone tubal ligation.

The incidence of HSV-2 infection was 6.8% among women with bacterial vaginosis (BV) as compared with 5.1% among women without BV and this association was not statistically significant. Similarly, the incidence was 6.7% among women with Trichomonas vaginalis infection as compared with 5.1% among those without infection. In addition, seroconversions were higher among women with low income, low age at first sex, and women who had partners with other sex partners, and none of these differences were statistically significant.

DISCUSSION

We enrolled and retained a large cohort of young reproductive age women from the rural and periurban communities of Mysore and found a moderate rate of HSV-2 seroconversions. Because of the small number of incident cases, we were unable to find any predictors of HSV-2 infection.
Previous studies in India had shown much higher rates of HSV-2 seroconversion among women attending STD clinics and HIV voluntary counselling and testing centers. Kumarasamy et al., in Chennai, conducted a study among women at high risk for HIV and found the prevalence of HSV-2 infection to be 50% and a 1-year incidence to be 8%.[3] Reynolds et al., in Pune, in a retrospective cohort study of 463 HIV-1 seronegative patients attending three STD and one gynecology clinic, found a prevalence of 50.9% among females with an STI and 89.1% among female sex workers yielding an incidence rate of 15.1/100 person-years and 33.8/100 person-years, respectively.[4] Our study found a prevalence of 13.2% and an incidence of 10.4/100 women-years for a community sample of women from urban and periurban neighborhoods in Mysore.

We did not find any associations with demographic or behavioral variables for incident infections. The patterns of association were similar to the findings from other studies, although our study had inadequate power to detect significant associations given the small number of seroconversions during the study period. As with previous studies, our study found a greater number of seroconversions among women with BV or T. vaginalis infection, low income, lower age at first sex, and women who had partners with other sex partners.[6]

The results of this study, while not statistically significant, still underline the need for managing lower genital tract infections, such as BV and T. vaginalis infection, because they have been shown to increase the risk for acquisition of viral infections, such as HSV-2 and HIV.[7–10] We had found HSV-2 prevalent infections to be associated with BV and T. vaginalis infections in this cohort.[11] More research is needed to better understand the biological mechanisms underlying these associations.

There are several limitations to this study. First, we did not do confirmatory testing for HSV-2 infection using Western blots as this procedure for HSV-2 infection is not available in India; so reported incidence might be overestimated. Even though our cohort was large, the low rate of HSV-2 seroconversions limited our ability to detect predictors of infection. This study with a follow-up period of 6 months may not be adequate to have enough seroconversions and may require longer duration of follow-up or a larger sample size. We did not include variables from the 3-month follow-up visit and limited our analysis to baseline covariates.

Despite those limitations, we were able to show that the incidence of infection is moderate in this large community sample of young reproductive age monogamous women. This study will help to understand the sample size needed or the study duration to examine hypotheses or study questions that require HSV-2 endpoints in India.

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Table 1

Characteristics\(^a\) of young reproductive age women in Mysore, India, and incidence per 100 women-years of follow-up

| Characteristics                   | Total with 1.1 cut-off | Incident HSV-2 Infections | Rate per 100 woman-years | P value |
|-----------------------------------|------------------------|---------------------------|--------------------------|---------|
|                                   | n  | %  | n  | %  |                  |          |
| Total                             | 696| 100| 36 | 5.2| 10.4               | 0.80     |
| Age categories (years)            |    |    |    |    |                    |          |
| 15–20                             | 49 | 7.0| 3  | 6.1| 12.2               |          |
| 21–25                             | 281| 40.4|13 | 4.6| 9.3                |          |
| 26–30                             | 366| 52.6|20 | 5.5| 10.9               |          |
| Years of education\(^b\)          |    |    |    |    |                    | 0.84     |
| 0                                 | 182| 26.1|10 | 5.5| 11.0               |          |
| 1–7                               | 195| 28.0|11 | 5.6| 11.3               |          |
| >7                                | 319| 45.8|15 | 4.7| 9.4                |          |
| Religion                          |    |    |    |    |                    | 0.73     |
| Hindu                             | 13 | 1.9| 0  | 0.0| 0.0                |          |
| Muslim                            | 473| 69.3|23 | 4.9| 9.7                |          |
| Christian                         | 210| 30.7|13 | 6.2| 12.4               |          |
| Monthly income (1 USD=48 INR)     |    |    |    |    |                    | 0.45     |
| 0–3999 INR                        | 535| 78 | 29 | 5.4| 10.8               |          |
| >4000 INR                         | 150| 22 | 6  | 4.0| 8.0                |          |
| Earns regular money               |    |    |    |    |                    | 0.38     |
| Yes                               | 198| 28.5|13 | 6.6| 13.1               |          |
| No                                | 497| 71.5|23 | 4.6| 9.3                |          |
| Have children                     |    |    |    |    |                    | 0.46     |
| Yes                               | 603| 86.6|33 | 5.5| 10.9               |          |
| No                                | 93 | 13.4| 3 | 3.2| 6.5                |          |
| Age at first sex (years)          |    |    |    |    |                    | 0.86     |
| <15                               | 122| 17.5| 8 | 6.6| 13.1               |          |
| 15–16                             | 208| 29.9|11 | 5.3| 10.6               |          |
| 17–18                             | 201| 28.9| 9 | 4.5| 9.0                |          |
| 19+                               | 165| 23.7| 8 | 4.8| 9.7                |          |
| Characteristics          | Total with 1.1 cut-off | Incident HSV-2 infections | Rate per 100 woman-years | P value |
|--------------------------|------------------------|---------------------------|--------------------------|---------|
|                          | n  | %  | n  | %  |                      |            |
| Husband has other sex partners |    |    |    |    |                      |            |
| Yes                      | 47 | 6.8 | 3  | 6.4 | 12.8                 | 0.02       |
| No                       | 353| 50.8| 13 | 3.7 | 7.4                  |
| Don’t know               | 295| 42.4| 19 | 6.4 | 12.9                 |
| Trichomonas vaginalis    |    |    |    |    |                      | 0.73       |
| Present                  | 46 | 6.6 | 3  | 6.7 | 13.0                 |
| Absent                   | 650| 93.4| 33 | 5.1 | 10.2                 |
| Bacterial vaginosis      |    |    |    |    |                      | 0.51       |
| Positive                 | 116| 17.4| 8  | 6.8 | 13.8                 |
| Intermediate             | 103| 15.4| 3  | 2.9 | 5.8                  |
| Negative                 | 449| 67.2| 23 | 5.1 | 10.2                 |

*a* Characteristics described are at the time of enrollment and none of the women included here had prevalent herpes simplex virus-2 infection.

*b* Totals differ because of missing data.