Original article

Seroprevalence of herpes simplex virus 2 infection among commercial sex workers in Colombo, Sri Lanka

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Abstract

Background: Herpes Simplex Virus Type 2 (HSV2) causes genital herpes, an incurable, lifelong sexually transmitted infection (STI). This study was conducted to describe the seroprevalence and associated factors of HSV2 infection in a cohort of Female Sex Workers (FSWs).

Methods: A descriptive cross-sectional study was conducted on 136 FSWs who attended the central STD Clinic, in Colombo. They were assessed using an interviewer-administered questionnaire followed by serum sampling. Serum samples were tested for HSV2 IgG using an enzyme immunoassay specific for glycoprotein gG2 of HSV2.

Results: HSV2 seroprevalence of FSW was 66.9% (91/136) of which only 14.3% (13/91) reported a history of genital infection. Age ≥35 years (OR 3.35, 95%CI 1.54-7.31, p=0.002), education ≥ grade 5 (OR 8.35, 95%CI 13.1-64.7, p<0.001), lifetime sexual partners ≥ 200 (OR 5.33, 95% CI 2.3-12.36), p<0.001), duration of sex work ≥1 year (OR 4.5, 95% CI 2.09-9.65, p<0.001) and history of STI (OR 4.52, 95% CI 1.9-10.78, p<0.001) were statistically significant risk factors for high HSV2 prevalence. Consistent condom use by commercial partners in the last 3 months was not a significant protective factor in preventing HSV2 infection (OR 0.44, 95% CI 0.15-1.26, p = 0.12) in this cohort.

Conclusions: The seroprevalence of HSV2 is high in this population and most of them did not report any genital infection. Multiple risk factors for higher prevalence were observed. Findings support the need for regular surveillance, monitoring of HSV-2 infection in high-risk populations, and expanding awareness of HSV-2 infection in the country.

Keywords: commercial sex workers, genital herpes, HSV-2 seroprevalence, HSV-2 IgG, Sri Lanka

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Introduction

Herpes Simplex Virus 2 (HSV-2) is sexually transmitted and causes genital herpes. It's a lifelong infection that can be treated with certain antivirals to reduce the severity of symptoms but is incurable due to its latency and recurrent nature.[1,2] Nearly 500 million people aged 15-49 worldwide are living with genital herpes.[3] Recurrent symptoms of genital herpes can lead to stigma, psychological distress, and a significant impact on quality of life, sexual relationships, and reproductive life. HSV-infected people are more prone to Human Immunodeficiency Virus (HIV) acquisition and neonates of infected mothers are at risk of neonatal herpes when exposed to HSV in the genital tract during delivery.[4,5]

Primary genital infection with HSV2 is characterized by widely spaced bilateral and painful lesions of varying stages, such as vesicles, pustules, or ulcers, on the external genitalia. In addition, many atypical symptoms occur in a significant proportion (60%) of patients and about 20% are asymptomatic.[6]

Ninety percent of persons who present with symptomatic first-episode genital HSV2 experience clinical reactivation of the disease with a median recurrence rate between four and five episodes per year.[7] Subclinical reactivation of the virus on mucus membrane is also common and 98% of those who had the symptomatic first episode experience subclinical HSV2 shedding in genital mucosa.[7] Chronic nature with reactivation and subclinical viral shedding leads to a high degree of transmission.

Detection of type-specific HSV-2 IgG in patients' blood is a standard serological method to diagnose genital herpes.[8,9] Seroprevalence of HSV2 varies by geographical location and population characteristics. Prevalence is highest in Sub-Saharan Africa and the Central and South Americas, lower in Western and Southern Europe than in Northern Europe and North America, and lowest in Asia but can vary due to age, country, region within the country, and population subgroup.[10]

A study done in Sri Lanka in 2000 showed a relatively higher prevalence of HSV2 in STD clinic attendees and a lower prevalence in low-risk populations (in antenatal mothers).[11] Since commercial sex workers are a high-risk category for acquiring and transmitting this infection, we aimed to identify the seroprevalence of HSV-2 and associated factors in this group of females.

Methodology

A descriptive cross-sectional study was conducted among female commercial sex workers (CSW) who attended the central STD clinic at the National STD and AIDS Control Program (NSACP) Colombo from January to April 2012. Females being more than 18 years of age and engaged in commercial sex for money or material for a month or more and consented by written informed consent to participate in the study were included. Those who were previously diagnosed with primary or secondary immunodeficiency including HIV (according to the history and previous records) and those who didn't give consent for obtaining information or a blood sample were excluded.

An interviewer-administered questionnaire was used to collect sociodemographic, clinical, behavioural, and sexual data. An interview was conducted by the principal investigator in a confidential room after providing all the information regarding the study and obtaining written consent. The patient identification details such as name, address, and workplace were not obtained and each person was assigned a serial number. Following the interview, the patient was directed to the bleeding room with the serial number labeled tube. From each participant, 3 ml of blood was obtained by a trained phlebotomist using a vacutainer tube and was sent to the microbiology laboratory at NSACP. Blood samples were centrifuged at 3000 rpm for 10 minutes at the microbiology laboratory and separated serum samples were stored in 2 ml cryovials at -70°C freezer until tested. Serum samples were thawed to room temperature and the HSV2 IgG test was performed using a type-specific commercial ELISA kit based on glycoprotein G2 specific for HSV2 (IBL International GMBH) according to the manufacturer's instructions.

Before assay, all samples were mixed well and diluted 1 in 100 with the sample diluent. 10µL sample and 1ml IgG sample diluent were dispensed into tubes and thoroughly mixed with a vortex. Positive, negative, and cut-off controls were mixed thoroughly and dispensed 100µL of each from B1 to E1, leaving A1 for substrate blank according to the instruction sheet. 100µL of diluted samples were dispensed into their respective wells. Wells were covered with the foil and incubated for one hour at 37°C. After completion of incubation, wells were washed 3 times using the washing solution in the ELISA washer. Then 100µL of HSV Type 2 anti-IgG conjugate was added to all wells except A1 and incubated for 30min at 37°C. After 30 minutes wells were washed as previously. 100µL TMB substrate solution was dispensed into each well including A1 and incubated for 30min at room temperature in the dark. Then 100µL of stop solution was added into all wells. Absorbance was measured at 450nm within 30 minutes using the ELISA Microwell plate reader. The test was validated by referring to the validation criteria given in the instruction sheet. Results were calculated and interpreted according to the formula given.

Data analysis was carried out using SPSS version 15. Descriptive statistics were done. Test results were cross-tabulated with socio-demographic and behaviour characteristics and the Chi-square test was performed as appropriate. Odds ratios with 95% confidence intervals and p values were obtained using the statistical package to meet the objectives. P ≥0.05 was taken as the level of significance.

Ethical clearance was obtained from the Ethical Review Committee, Medical Research Institute, Colombo (Project number 16-2011). Permission from the director, NSACP was obtained to carry out the study. Informed written consent was obtained from all female commercial sex workers for the interview and for collecting blood samples.

Results

A total of 140 CSWs presented to NSACP during the study period and 136 (97.1%) were eligible to participate in the study. Among the 136 CSWs, 91 were positive for HSV2-IgG with a seroprevalence rate of 66.9%. Out of the seropositive group (91), only 14.3% (13) reported the presence of painful genital lesions in the past, while 82.4% (75) did not reveal any evidence of clinical disease and 3.3% (03) couldn't recall. Within the seronegative group (45), only 2.2% (01) have reported genital ulcers/vesicles. The odds ratio of the presence of genital lesions among the seropositive and seronegative CSW groups is 7.3, 95% CI (1.03-57.96), and is statistically significant (p-value = 0.03).

Table 1 describes the population's sociodemographic characteristics and the seroprevalence of the infection. It clearly showed that the seroprevalence increases with advancing age and it is high in those who have not attended school or those who attended school only up to lower grades.

Table 1. Socio-demographic characteristics and HSV2 IgG positivity of participants

	CSW		*HSV-2 IgG positivity*	
	N	*%*	*No. positive*	*% positive*
Age				
15-24	25	9.3	13/25	52
25-34	49	18.3	28/49	57.1
35-44	34	12.7	26/34	76.5
45-54	25	9.3	21/25	84
55-64	1	0.4	1/1	100
>64	2	0.7	2/2	100
Marital status				
Married	43	16.0	24/43	55.8
Divorced/widowed/separated	85	31.7	62/85	72.9
Never married	8	3.0	5/8	62.5
Education				
No schooling	16	11.8	15/16	93.8
1-5	21	15.4	19/21	90.5
6-10	55	40.4	34/55	61.8
Up to O/L	37	27.2	19/37	51.4
Up to A/L	7	5.1	4/7	57.1
University	0	0	0	0
Ethnicity				
Sinhala	111	81.6	71/111	64
Tamil	18	13.2	13/18	72.2
Muslim	6	4.4	6/6	100
Other	1	.7	1/1	100

Table 2 describes the use of substances during the last 12 months, and it is observed that the seroprevalence of HSV-2 was highest in those who use narcotics. The number of lifetime commercial sexual partners in this population ranged from <10 to >1000 and the seroprevalence has drastically increased with the increasing number of lifetime sexual partners. The mean age of coitarche in CSWs was 18.74 years and 40% of CSWs had coitarche at <18 years.

The majority of the CSW (49.3%,67) were referred to the STD clinic by the courts for screening of Sexually Transmitted Infections (STI) and another 32.3%, (44) came voluntarily for STI screening (Figure 1). A minority of them (18.4%, 25) have presented with symptoms of STI with 10% (14) having vaginal discharge (Figure 1).

According to this study age ≥35 years, education < grade 5, duration of sex work ≥1 year, lifetime sexual partners ≥200, and presence of an STI in the past were significant risk factors for HSV2 infection at 5% level. But at 10% level, being divorced/widowed/separated and substance abuse in the last year were also risk factors for HSV2, with p values of 0.06 and 0.069 respectively (Table 3 and 4).

Out of 136 CSWs 81.6%, (111) had always used condoms with commercial partners during the last 3 months, but 18.4% (25) did not use condoms consistently for the past 3 months. The majority of CSW (64.7%, 88) had one or more regular partners in the last 3 months and 78.4% (69) of them never used condoms with these regular partners. Consistent condom use within the last 3 months by the commercial partner was not a significant protective factor in this study. The presence of a regular partner in the last 3 months was a significant protective factor.

Table 2. Behavioural characteristics and HSV2 IgG positivity of two study populations

	CSW		*HSV-2 IgG positivity*	
	N	*%*	*No*	*%*
Substance use last 12 months				
None	91	66.9	56/91	61.5
Alcohol	35	25.7	27/35	77.10
Tobacco	3	2.2	2/3	66.7
Narcotics (inhalation/oral)	7	5.2	6/7	85.7
Age at first sexual encounter (coitarche)				
12-17	54	39.7	14	11.4
18-24	67	49.3	67	54.5
25-35	15	11.0	41	33.3
35-45	0	0	0	0
Number of lifetime commercial sexual partners				
<10	21	15.4	5/21	23.8
10-50	19	14.0	11/19	57.9
51-100	13	9.6	6/13	46.2
101-200	22	16.2	17/22	77.3
201-500	17	12.5	13/17	76.5
501-1000	24	17.6	20/24	83.3
>1000	20	14.7	19/20	95

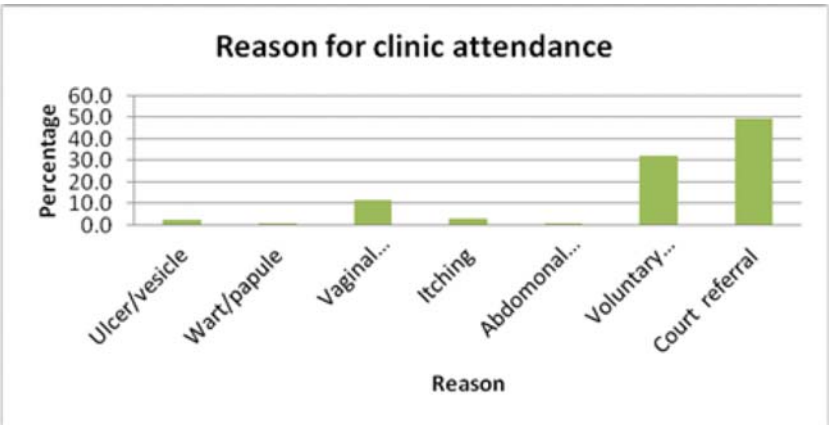

Figure 1. Reason for attending STD Clinic.

Table 3. Risk factors for HSV2 infection in CSW

Variable	*Test Positive %*	*Test Negative %*	*OR (95% CI)*	*P value*
Age				
<35 years	55.4	44.6	3.35 (1.54-7.31)	0.002
≥35 years	80.6	19.4		
Marital status				
Married	55.8	42.2	2.04 (0.96-4.33)	0.06
Div/Wid/Never married	72	28		
Education				
≤ grade 5	91.9	8.1	8.35 (2.41-29.2)	0.001
> grade 5	57.6	42.4		
Substance abuse during last 12 months				
Yes	77.8	22.2	2.13 (0.93-4.83)	0.069
No	61.5	38.5		
Age at first sexual encounter				
<18	59.3	40.7	0.55 (0.26-1.14)	0.105
≥18	72	28		
Duration of commercial sex				
<1 year	48.3	51.7	4.5 (2.09-9.65)	0.001
≥1 year	80.8	19.2		

Table 4. Risk factors for HSV2 infection in CSW

Variable	*Test Positive %*	*Test Negative %*	*OR (95% CI)*	*P value*
Lifetime commercial sexual partners				
<200	52	48	5.33 (2.3-12.36)	0.001
≥200	85.2	14.8		
Commercial sexual partners last 3 months				
<50	63.4	36.6	1.67 (0.75-3.75)	0.21
≥50	74	26		
Condom use by commercial partner last 3 months				
Always	63.4	36.4	0.44 (0.15-1.26)	0.12
Sometimes/ Never	74	20		
Presence of regular partners				
Yes	60.2	39.8	0.40 (0.18-0.90)	0.025
No	79.2	20.8		
History of STI				
Yes	84.9	44.6	4.52 (1.9-10.78)	0.001
No	55.4	15.1		

Discussion

The seroprevalence of HSV2 among the CSW population was 66.9% in this study. Very few (14.3%) have reported genital infection, and the majority (82.2%) did not reveal any evidence of clinical genital infection. This showed a high percentage of unrecognized and asymptomatic HSV2 infection among this cohort which may lead to the asymptomatic spreading among sexual contacts. A study done in the year 2000 in STD clinic attendees in Ragama, Sri Lanka showed HSV2 seroprevalence of 49.3% and 33% in females and males respectively.[11] But in that study seroprevalence in sex workers was not analysed separately. Similar rates were reported among FSWs in Yannan province, China which was 68.1%.[12] Higher HSV2 prevalence rates were detected in FSW populations from other Asian countries, 80% in Japanese sex workers,[13] 86.2% in Korean sex workers,[14] 94.7% in brothel-based female CSW in Bangladesh[15] and 85.2% in STD clinic attendees in New Delhi, India.[16] A study conducted in five STD clinics in America has shown that 84.7% of HSV2 seropositive patients had never been given a clinical diagnosis of genital herpes[17] which is similar to our findings. The high proportion of unrecognized HSV2 infection in the seropositive group suggests that clinical diagnosis and the reported history of genital herpes will not identify the majority of the individuals with HSV2 infection but, there is potential spread due to recurrent nature and asymptomatic spreading. Therefore, HSV2 serological testing should be considered an important test in diagnosing infection in high-risk populations.

According to this study, advancing age, low education, increased number of lifetime sexual partners, duration of sex work >1 year, and history of STI are significantly associated with increased prevalence of HSV-2 infection and are significant risk factors. These findings are similar to previous studies done in FSW and in STD clinic attendees in different countries.[12,16,17,18]

Consistent condom use by commercial partners within the last 3 months has been associated with decreased

rates of HSV2 in CSW, however, it was not a significant protective factor for HSV2 infection in this study. As this is a cross-sectional study, the relationship between condom use and HSV2 was not explored prospectively and information from the last 3 months was gathered to avoid recall bias, but HSV2 could have been acquired at any time after coitarche. Other reasons for the lack of association in this study may be related to incorrect condom usage leading to condom slipping off or breakage, use of poor quality condoms leading to breakage, inaccurate self-reporting, and the presence of viral shedding sites that are not covered by condoms. Similar results were also reported from previous studies done among high-risk populations.[11,18] Preventive measures such as consistent and proper use of condoms, education, and awareness among the risk groups and the general public, routine screening is important in preventing infection.

Conclusion

Seroprevalence of HSV2 is very high among FSWs in the Colombo district. A majority of positive CSWs did not have clinical genital infection, but they are potential sources of infection. Statistically significant risk factors for HSV2 infection in FSWs in this study are age ≥35 years, education ≥ grade 5, lifetime sexual partners ≥200, duration of sex work ≥1 year, and presence of other STIs in the past. Regular condom use with commercial partners in the last 3 months was not a significant protective factor for the prevention of HSV2 infection in this study. However, there was a lower HSV2 seroprevalence in those who have used condoms regularly compared to those with inconsistent use. Routine screening of high-risk populations to identify those infected, enhancing education and awareness of high-risk groups and the public in the country, and strengthening preventive measures are important to minimize the acquisition of new infections.

Author contribution

Developed the research concept and preparation of the first draft: DN, Supervision of data collection, testing: GG, SM, Collection, and verification of data, testing, and test verification: DN, Literature Search: DN, Writing of the manuscript: DN, Final revision and editing: GG, SM.

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