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Prevalence and predictors of herpes simplex virus type 2 infection among female sex workers in Yunnan Province, China

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Summary

The objective of this study was to determine the seroprevalence of herpes simplex virus type 2 (HSV-2), and to evaluate the relationship between HSV-2 infection and sociodemographic factors and the sexual practices of female sex workers (FSWs) in Kaiyuan city, Yunnan Province, China. This cross-sectional study involved 737 FSWs and was carried out from March to May 2006 with confidential interviews and laboratory tests for HSV-2 and other sexually transmitted infections (STI). HSV-2 was the most common STI (68%), followed by *Chlamydia trachomatis* (26%), *Trichomonas vaginalis* (11%), *Neisseria gonorrhoeae* (8%) and syphilis (7%). Prevalence of HIV-1 was 10.3%. Adjusted odds ratios of HSV-2 seroprevalence were 2.6 (95% CI [confidence interval]: 1.30–5.38) for HIV-1 infection, 2.0 (95% CI: 1.33–3.16) for vaginal douching, 2.0 (95% CI: 0.45–0.86) for condom breaking or falling off during sexual intercourse with the client in the previous week, 1.8 (95% CI: 1.07–3.18) for ≥ 5 years of commercial sex work, 1.6 (95% CI: 1.08–2.33) for ≥ 5 clients in the previous week, 0.6 (95% CI: 0.45–0.86) for ≥ 9 years of education. This study identifies a very high prevalence of HSV-2 infections among FSWs in Yunnan Province, with only a few who reported a prior history of genital herpes. HSV-2 serological screening and suppressive therapy should be considered for study populations. Education on the importance of diagnosis, treatment and prevention may help control the spread of HSV-2 infection.

Keywords

herpes simplex virus type 2 (HSV-2); female sex worker; condom; sexually transmitted infection; China

INTRODUCTION

Herpes simplex virus type 2 (HSV-2) infection is one of the most common sexually transmitted diseases and the most common cause of genital ulcer disease in the world.^{1,2} This is a problem

in China because since the 1980s, commercial sex work has become increasingly prevalent. By 2000, the Chinese Public Security office estimated four to six million sex workers nationwide, a 160-fold increase since 1985.³ However, little data are available about the prevalence of HSV-2 among female sex workers (FSWs) in China.

Genital herpes infections have been associated with increasing human immunodeficiency virus type 1 (HIV-1) infection.^{4–8} A recent meta-analysis showed that patients who were seropositive for HSV-2 had three times the risk of acquiring HIV-1, when compared with those who were seronegative for HSV-2.⁹ Even in the absence of active genital ulcer disease, there is a correlation between genital tract shedding of HIV-1 and HSV-2.^{10,11}

HIV infection rates are highest in Yunnan Province in southern China.¹² Although the HIV epidemic has been driven there predominantly by injection drug use, sexual transmission now plays an increasingly greater role¹³ and thus, research about HSV-2, is particularly important because of its role in facilitating the transmission of HIV. The goal of this study is to determine relationship between HIV and HSV-2 in FSWs in a high-risk area of China and to describe the risk factors for HSV-2 infection in this group. This study was conducted in Kaiyuan City, Yunnan with a population of 292,000 and a large numbers of sex workers.

METHODS

This study was conducted by the Chinese Center for Disease Control and Prevention (China CDC) in Beijing with provincial and local CDC staff in Yunnan. After approval from the China CDC and Yunnan CDC institutional review boards, we conducted a cross-sectional study of all women identified as FSWs in Kaiyuan City, Yunnan Province from March to May 2006. The inclusion criteria for the study were women ≥ 16 years old, self-reported to have sold sex for money within the previous three months, and agreed to be tested and counseled for HIV, other STIs, and for the use of illegal drugs. After providing voluntary informed consent, participants were asked standard questions about their demographics, basic medical history and behavioural risk factors.

Blood was collected and tested for HSV-2 antibody (HerpeSelect-2 ELISA IgG, Focus, USA), syphilis (rapid plasma reagin [RPR] test, Xinjiang Xindi Company, China) and HIV-1 antibody (enzyme-linked immunosorbent assay [ELISA], Vironostika HIV Uni-Form plus O, bioMerieux, Holland). Positive HIV-1 ELISAs were confirmed by western blot (Diagnostics HIV Blot 2.2, Genelabs, USA) and positive RPR tests were confirmed by the *Treponema pallidum* particle assay (TPPA) test (Serodia-P-PA-Fujirebio, Fuji, Japan). Endocervical swabs were collected and tested for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* by polymerase chain reaction (PCR, AMPLICOR, Roche, USA). Vaginal swabs were collected and a wet-mount was prepared to detect *Trichomonas vaginalis*. Finally, urine was collected for opiate screening (MOP One Step Opiate Test Device, ACON Laboratories, Inc., USA). Participants were classified as using illegal drugs if they either self-reported such use or tested urine positive for opiates. All participants were scheduled for a follow-up visit four to six weeks later for post-test counselling.

Statistical tests were performed using SASTM 9.1 software (SAS Institute Inc, Cary, NC, USA). Chi-square tests were used to compare illegal drug users and non-drug users. The correlations between HSV-2 seroprevalence and age, duration of commercial sex work, and the number of clients in the previous week were evaluated with the chi-square test for linear trend. The consistency between self-report and urine screening for drug use was tested using Kappa coefficient. Univariate associations between risk factors and HSV-2 seropositivity were evaluated using simple logistic regression analysis. Adjusted odds ratios (OR) were calculated by including factors with $P < 0.2$ in univariate analyses and then by limiting final multivariate

models to risk factors or confounders that were statistically significant ($P \leq 0.05$) in the overall analysis. Higher sex worker risk venues were defined as locations where FSWs generally charged less than 100 Yuan (about \$13 USD) for sex services, including beauty salons, temporary sublets, and street walkers; these FSWs attracted the less wealthy and less educated clients potentially at higher risk of HIV infection, such as drug users. Lower sex worker risk venues were defined as locations where FSWs generally charged 100 Yuan or more for sex, including karaoke clubs, night clubs, saunas and hotels.

RESULTS

Demographics of the study population

Of the 756 FSWs screened for the study, 15 were excluded because they had not sold sex for money in the previous three months, and four others were excluded because specimens could not be obtained. All analyses were based on the final 737 FSWs.

Table 1 describes the univariate relationship between HSV-2 positivity and demographic and behavioural characteristics of the FSWs studied. Ages ranged from 16 to 52 years (median, 23 years; inter-quartile range [IQR], 20–28 years). Older women subjects working in higher risk venues, and women living in brothels were significantly more likely to be HSV-2-positive. Women with ≥ 9 years of formal education were significantly less likely to be infected. The linear model, applied to the increase in seroprevalence with age, was not rejected by chi-square statistics for linearity, implying a dose-response relationship between age and HSV-2 infection risks.

Prevalence of HIV-1 and other sexually transmitted infections

Over two-thirds of subjects were infected with HSV-2 (Table 2). Prevalence of the other STIs ranged from 7.5% to 25.9%, with HIV-1 being 10.3%. A total of 583 (79%) of the 737 participants were positive for one STI (not including HIV), 176 (24%) had two concurrent infections and 62 (8%) had three. Subjects with syphilis or HIV-1 infection had a significantly higher seroprevalence of HSV-2 infection than those without.

There was no significant association between HSV-2 infection and a history of lower abdominal pain, unusual vaginal discharge, pain or burning on urination, or genital ulcers during the previous year. Only 11 HSV-2 seropositive subjects (2.2%) reported a history of genital herpetic ulcers in the previous year, and three (0.6%) reported a current ulcer.

Behavioural characteristics and association with HSV-2 infection

Table 3 describes the relationship between HSV-2 infection status and several behavioural characteristics. Illegal drug users had a 3.6-fold greater risk of HSV-2 infection. A total of 120 FSWs (16.3%) either self-reported illegal drug use or had a positive urine drug test. Of the 114 FSWs who tested urine opiate positive, 82 (72%) of them self-reported. Correspondingly, of the 88 FSWs who reported any illegal drug use, 82 (93%) tested urine opiate positive. The Kappa test showed that the results of self-reporting and urine testing had very good consistency (Kappa = 0.78, 95% CI, 0.72–0.85, $P < 0.0001$). Among all FSWs, the median duration of commercial sex work was 19.4 months (for HSV-2-negative, 14.6 months; HSV-2-positive, 22.2 months), and the median number of paid clients in the preceding week was three (two vs. three for HSV-2-negative and -positive subjects, respectively).

Fifty-two percent of women reported a 'regular partner', defined as a male with whom she had regular sexual relations without compensation, such as a boyfriend or spouse. There was no association between using condoms with regular sex partners and being HSV-2 seropositive. In contrast, the overall seroprevalence of HSV-2 was significantly lower in those reporting

consistent condom usage with clients (66.1% vs. 78.5% for those not always using condoms with clients, $P = 0.0073$). Of the 708 subjects who said they had used condoms with at least one client in the preceding week, 60 (8.5%) said that a condom broke or fell-off during intercourse; these women had a significantly higher HSV-2 seroprevalence (81.7% vs. 66.5% for those not reporting condoms breaking or falling off, $P = 0.0162$).

Vaginal douching was very common in these FSWs (84.1%). The overall seroprevalence of HSV-2 was significantly higher in those who douched (70.2% vs. 57.3% for those not practicing vaginal douching, $P = 0.006$). Medical disinfectant (91.9%) was the most commonly reported douche solution, followed by water alone (61.0%), toothpaste (31.3%), saline water (17.7%), vaginal spermicide (3.9%), soap (3.4%) and vinegar (1.0%).

Multivariate analyses

Table 4 displays the results from multivariate logistic regression models of HSV-2 seroprevalence. After adjusting for the risk and host factors significant in univariate analyses, the following remained statistically significant risk factors for HSV-2 infection: increased duration of sex work, increased numbers of clients in the previous week, history of vaginal douching and testing positive for syphilis or HIV-1. Increased education was associated with a significantly lower risk of HSV-2.

DISCUSSION

The high prevalence of HSV-2 infection identified in our study (68.1%) is similar to rates reported elsewhere among FSWs in Asia – from 5% in Taiwan,¹⁴ 35% in Bangladesh,¹⁵ 65% in Kunming, Yunnan Province,¹⁶ 71% in Korea,¹⁷ 76% in Chiang Rai,¹⁸ to 80% in Japan.¹⁹ Our results further show that HSV-2 is highly prevalent in FSWs in this area of China, with longer duration of employment and increased numbers of clients being significantly at risk for HSV-2 infection when key variables are controlled. This suggests that the numbers of partners per week and the numbers of years in sex work are the key sexual predictors of HSV-2 infection, and that older women are more likely to be positive for this incurable infection because of their greater exposure to sexual situations where the virus can be transmitted. In terms of socioeconomic characteristics, women with less education were at a greater risk of infection with HSV-2. These findings are consistent with the results of other studies.^{20,21} Less education may lead to lower knowledge of STD and STD prevention, and may be associated with less frequent or less-proficient condom use.

Although condom use was not a significant risk factor for HSV-2 infection of the sex workers in our study, consistent condom usage has been associated with decreased rates of HSV-2 infection.²² The reasons for the lack of association in our study may be related to inaccurate self-reporting, poor quality of the condoms leading to breakage, incorrect condom usage leading to condoms slipping off or overall condom usage that was not consistent enough to prevent transmission. Although 80% of HSV-2-infected FSWs said they generally used condoms with clients, condoms were rarely used with boyfriends and spouses, allowing for continued transmission of HSV-2 and other STIs. Our finding of high rates of STIs corroborates this continued transmission cycle between FSWs and their clients and regular unpaid sex partners. These findings suggest that frequent screening and treatment of FSWs for HIV, HSV-2 and other STIs should be encouraged, as well as educational programmes promoting consistent and correct condom usage with all sexual partners, not just paid clients. These results are consistent with other studies of FSWs in China which suggest that they are a major reservoir for expanding STIs and HIV in China.²³

The risk of HSV-2 seroprevalence was higher among FSWs with syphilis infection. A comparison of the unadjusted OR of 5.1 of HSV-2 for syphilis (Table 2) to the adjusted OR of

5.3 for syphilis (Table 4) shows that adjusting for these key sexual exposure risk factors had little effect on the OR for syphilis and, if anything, only increased it. Other studies have also identified syphilis as a risk factor for HSV-2.^{24–26} Whether this reflects an interaction that facilitates co-infection or just represents a subgroup at higher risk for both infections is unclear. It may be owing to the genital ulcers caused by syphilis, which provide a portal of entry for HSV-2. However, HSV-2 and syphilis are both ulcerative STIs that are known to be important cofactors in the transmission of HIV.

Our results also demonstrated that HIV-1 infection was independently correlated with HSV-2 infection when adjusted for the major sexual risk factors in this study: duration of sex work and number of clients in the week before the interview. Biological and epidemiological studies suggest potential bidirectional interactions between HSV-2 and HIV-1. Genital ulcers may provide a portal of entry for HIV-1,²⁷ as well as facilitate the transmission of HIV-1 by increasing the HIV-1 viral load in the blood, plasma, semen and HSV-2 genital ulcers.²⁸ HIV may increase susceptibility to HSV-2 infection and genital shedding, HSV-2 recurrence rate and severity of clinical manifestations. It may be because of the immunosuppression associated with HIV-1 disease, or it could occur under the influence of other systemic or local cofactors of HIV-1 genital shedding, as one previous study demonstrated increased shedding of HSV-2, associated with declining CD4 T cell counts, among HIV-1-seropositive women in New York City.²⁹ Suppressive therapy of HSV-2 has been shown to reduce genital and plasma HIV RNA levels.^{30,31} Studies are underway to determine if the treatment of HSV-2 will reduce HIV transmission.³²

Vaginal douching was practiced by 84% of the FSWs in our study. We found that those who douched were at two-fold greater risk of HSV-2 infection after adjusting for other key risk factors. Douching may remove normal vaginal flora, permitting the overgrowth of pathogens. It may also provide a pressurized fluid vehicle for pathogen transport, helping lower genital tract infections, ascend above the cervix into the uterus, fallopian tubes or abdominal cavity.³³

Our study has several limitations. Because some of the data collected was based on self-report, some risk factors, such as the use of condoms and illegal drug usage may be misclassified. To minimize recall bias, we asked questions about recent events, such as condom use in the previous week, and conducted objective tests of risk factors as much as possible, such as urine testing for opiates. The Kappa testing showed that measures of illegal drug use based on self-report and urine tests were consistent.

Importantly, over 97% of the HSV-2 seropositives did not report a history of genital herpes and 99% had no clinical signs of infection on physical examination although tested positive for HSV-2. The high proportion of unrecognized HSV-2 infections suggests that clinical diagnosis and reported history of genital herpes will not identify the majority of the individuals with HSV-2 infection. HSV-2 serological screening programmes should be considered in select population groups at especially high risk of infecting others.

The high seroprevalence of HSV-2 infection found in the FSWs surveyed has implications for public health in Yunnan and possibly in China and other areas because FSWs could be a significant source of infection for the local heterosexual population. Since most HSV-2 infections are asymptomatic, serological screening is necessary to identify those infected. The very high seroprevalence of HSV-2 in FSWs is of particular concern because genital ulcers caused by HSV-2 facilitate HIV transmission. Suppressive therapy may be indicated in some seropositive FSWs, especially those co-infected with HIV, irrespective of whether they have clinical symptoms. Proper use of condoms and education on the importance of diagnosis, treatment and prevention may help control the spread of HSV-2 infection.

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Table 1
Demographic factors associated with herpes simplex virus type-2 (HSV-2) seropositivity among 737 female sex workers (FSWs)

Factors	No. FSWs	HSV-2 positive (row %)	OR (95% CI)
Age (years)			
16–20*	225	136 (60.4)	
21–25	255	178 (69.8)	1.5 (1.04–2.21)
26–52	257	188 (73.2)	1.8 (1.21–2.62)
Nationality			
Han*	492	325 (66.1)	
Other	245	177 (72.2)	1.3 (0.96–1.87)
Registered permanent residence			
Outside Yunnan*	145	93 (64.1)	
Kaiyuan city	297	198 (66.7)	1.1 (0.74–1.70)
Other cities in Yunnan	295	211 (71.5)	1.4 (0.92–2.15)
Education level			
<9 years*	387	282 (72.9)	
≥9 years	350	220 (62.9)	0.6 (0.46–0.86)
Marital status			
Single or cohabitation*	516	351 (68.0)	
Married, separated, divorced or widowed	221	151 (68.3)	1.0 (0.72–1.42)
Entertainment venue			
Lower risk*	458	294 (64.2)	
Higher risk	279	208 (74.6)	1.6 (1.17–2.27)
Residence type			
Apartment	491	314 (64.0)	
Family home	59	44 (74.6)	1.7 (0.90–3.06)
Brothel or other working location	187	144 (77.0)	1.9 (1.28–2.78)

* Reference group

Table 2

STIs and relevant symptoms associated with HSV-2 seropositivity among 737 FSWs

Type of infection and percent of FSWs infected (95% CI)	STI infection status (no. tested)	HSV-2 positive no. (%)	Unadjusted OR of HSV-2 (95% CI)
HSV-2 68.1 (64.6–71.5)	No 682 [†]	452 (66.3)	
Syphilis 7.5 (5.7–9.6)	Yes 55	50 (90.9)	5.1 (2.00–12.93)
<i>N. gonorrhoeae</i> 8.3 (6.4–10.5)	No 676 [†]	460 (68.1)	
<i>C. trachomatis</i> 25.9 (22.8–29.2)	Yes 61	42 (68.9)	1.0 (0.59–1.83)
<i>T. vaginalis</i> 10.6 (8.5–13.0)	No 546 [†]	370 (67.8)	
HIV-1 10.3 (8.2–12.7)	Yes 191	132 (69.1)	1.1 (0.75–1.52)
	No 659 [†]	444 (67.4)	
	Yes 78	58 (74.4)	1.4 (0.82–2.39)
	No 661 [†]	436 (66.0)	
	Yes 76	66 (86.8)	3.4 (1.72–6.75)

[†]Reference group

Table 3
Behavioural characteristics associated with herpes simplex virus type-2 (HSV-2) seropositivity among 737 female sex workers (FSWs)

Characteristics	No. FSWs	HSV-2 positive, no. (%)	Unadjusted OR of HSV-2 (95% CI)
Tooth extracted or filled			
No [*]	614	415 (67.6)	
Yes	123	87 (70.7)	1.2 (0.76–1.77)
Blood transfusion			
No [*]	714	486 (68.1)	
Yes	23	16 (69.6)	1.1 (0.44–2.64)
Illegal drug user			
No [*]	617	398 (64.5)	
Yes	120	104 (86.7)	3.6 (2.06–6.21)
Age of first sex (years)			
<18 [*]	450	300 (66.7)	
≥18	287	202 (70.4)	1.2 (0.86–1.64)
Starting age in commercial sex (years)			
<20 [*]	266	178 (66.9)	
≥20	471	324 (68.8)	1.1 (0.79–1.50)
Duration of commercial sex work (years)			
<1 [*]	248	148 (59.7)	
1–2	284	196 (69.0)	1.5 (1.05–2.15)
3–4	97	70 (72.2)	1.8 (1.05–2.92)
≥5	108	88 (81.5)	3.0 (1.72–5.14)
Number of clients previous week			
1 [*]	190	110 (57.9)	
2–4	330	225 (68.2)	1.6 (1.08–2.26)
≥5	217	167 (77.0)	2.4 (1.58–3.73)
Always used condom with regular partner in previous week			
Yes [*]	61	40 (65.6)	
No	322	212 (65.8)	1.0 (0.57–1.80)
Always used condom with clients in previous week			
Yes [*]	616	407 (66.1)	
No	121	95 (78.5)	1.9 (1.18–2.99)
Condom broke or fell off during sex with clients in previous week			
No [*]	648	431 (66.5)	
Yes	60	49 (81.7)	2.2 (1.14–4.40)
Vaginal douching			
No [*]	117	67 (57.3)	
Yes	620	435 (70.2)	1.8 (1.17–2.63)

* Reference group

Table 4

Multivariate analyses of risk and protective factors of herpes simplex virus type-2 (HSV-2) infection among 737 female sex workers (FSWs), based on the logistic regression model*

Risk factors	Adjusted OR (95% CI)	P value
Duration of commercial sex work ≥ 5 years (compared with duration < 1 year)	1.8 (1.07–3.18)	0.0268
Number of clients in previous week ≥ 5 (compared to 1 client)	1.6 (1.08–2.33)	0.0186
Education level ≥ 9 years	0.6 (0.45–0.86)	0.0011
Vaginal douching	2.0 (1.33–3.16)	0.0011
Condom breaking or falling off during sexual intercourse with client in previous week	2.0 (1.00–4.15)	0.0489
Syphilis infection	5.3 (2.02–13.64)	0.0007
HIV-1 infection	2.6 (1.30–5.38)	0.0074

* To reduce residual confounding, years of education, duration of sex work and number of clients in the preceding week were entered into models as continuous variables. The following odds ratios and 95% confidence intervals were found for these risk factors: per increased year of education: 0.9 (0.87–0.98); per additional client: 1.1 (1.02–1.11) and per additional year in sex work: 1.1 (1.03–1.18)