

Analysis of Herpes simplex virus 1 and 2 infection in women with high risk sexual behaviour in Mexico

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Background	This paper describes the seroprevalence and risk factors of Herpes simplex virus (HSV) infection in a group of female prostitutes from Mexico City.
Methods	Women who consented to participate in the study voluntarily attended a sexually transmitted disease (STD) clinic during 1992. A standardized questionnaire was administered and a blood sample was obtained from each participant. Type-specific Western blot serology was performed to determine the serostatus of HSV-1 and HSV-2 for participants. Bivariate and multivariate analyses were applied to identify variables associated with an increased risk for HSV infection.
Results	Prevalences of infection among the 997 prostitutes studied were 93.9% for HSV-1 and 60.8% for HSV-2. Only 1.8% of the women were seronegative for both viruses. The only variable associated with HSV-1 seropositivity was crowding index. The following variables were associated with an increased risk for infection with HSV-2: age, level of education, working site, born outside Mexico City and increasing time as a prostitute.
Conclusions	This is the first assessment of HSV infection in Mexico and may be useful for the development and application of control and preventive measures among the prostitute population at risk of acquiring and transmitting human immunodeficiency virus (HIV) and other STD.
Keywords	Herpes virus infection, female prostitutes, Mexico City
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Herpes simplex virus (HSV) types 1 and 2 are closely related viruses belonging to the family *Herpesviridae*. Both types establish latent infections in humans which periodically reactivate and may be associated with recurrent episodes of disease.^{1,2} Clinically, the presence of oral and genital ulcers may result in painful lesions, as a consequence of primary HSV-1 or 2 disease.³ The majority of HSV infections are asymptomatic; the virus is periodically shed in both symptomatic and asymptomatic infections, and as such the infection may often be transmitted without knowledge.^{4–7} One of the most serious consequences of unrecognized HSV-2 infection is the potential for infecting neonates as they pass through the birth canal. Such an infection may cause fatal or neurologically damaging encephalitis, severe or fatal disseminated forms of the disease and ocular sequelae as well.¹

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Genital herpes is a sexually transmitted disease (STD) that can develop following infection with either HSV-1 or HSV-2. The acquisition of HSV-1 is by oral-to-genital contact, occurring mostly as a primary infection, with a lesser frequency of genital recurrences than HSV-2. In the case of the latter it is genital-to-genital or genital-to-anal contact that renders virus transmission.³

In terms of the estimated number of HSV-2 cases in the US, recent data from NHANES III (1988–1994)⁸ show that there are 45 million people infected, with an increase of 30% in viral seroprevalence, compared with NHANES II (1976–1980).⁹ Hence seropositivity to HSV-2 is currently recognized in nearly one in five people ≥ 12 years of age in the country.⁸

There is considerable homology in nucleic acid sequence between HSV-1 and HSV-2; around 40% of their DNA sequences are highly conserved.¹⁰ This results in an antigen-sharing profile of the two serotypes. Consequently, antibodies produced in response to one type exhibit extensive cross-reactivity with analogous proteins of the other type, making traditional serological approaches difficult and inconclusive.¹¹ This is particularly true for individuals who have been exposed to both HSV-1

and HSV-2. Thus, the most effective means for identifying asymptomatic herpesvirus infections is through type-specific serology.⁹

Glycoprotein G is the only known protein encoded by HSV-1 and HSV-2 that shares no sequence homology between the two viruses, apart from a short membrane anchor and leader sequence.^{12,13} Its isolation and purification have greatly improved the reliability of type-specific HSV detection, and assays based on glycoprotein G (gG-1 and gG-2) have been used in a variety of epidemiological studies.¹⁴⁻¹⁶

Worldwide, there has been renewed interest in HSV genital infection stemming from the observation that, together with syphilis and chancroid, genital HSV infection may increase the risk for HIV infection.¹⁷⁻²⁰ This risk is much greater with genital herpetic ulcers, and with their recurrences, than syphilitic or chancroid ulcers. In this article we present a seroprevalence survey of HSV type-specific antibodies for gG-1 and gG-2 among women with high-risk sexual behaviour. We also evaluated risk factors associated with herpes infection in this population of prostitutes.

Materials and Methods

Study subjects and sampling procedures

After obtaining informed consent, blood samples were taken from 1000 female prostitutes who voluntarily sought medical attention and HIV testing at an Information Center of Mexico's National Council for AIDS Prevention and Control (CONASIDA). The yearly average number of attendees to this clinic in Mexico City is 2480.²¹ The 1000 women included in the study were all those who attended between January and October 1992 from 9 a.m. to 12 p.m. They were working in sites requiring the use of a card provided by CONASIDA that certifies their serological status for HIV. Herpes virus testing was offered only for research purposes. The sample size was determined by the reagents available to perform laboratory testing.

Ten ml of peripheral blood was obtained by venipuncture using Vacutainer SST tubes (Becton-Dickinson, Rutherford, NJ). After allowing clot retraction at room temperature, the tubes were centrifuged at 1500 g for 10 min. Sera were placed in vials labelled with bar codes and refrigerated at 4°C for no longer than a week before type-specific HSV and syphilis serological tests were performed. Afterwards, the remaining sera were frozen at -20°C.

All women completed a questionnaire with three sections which was administered by trained female social workers. The first section covered socioeconomic and demographic characteristics such as age, education, marital status, place of birth; housing characteristics (e.g. number of bedrooms, number of people in the household); possessions (e.g. video cassette players, car, refrigerator and washer); and type of work site (e.g. street or bar). The second section was used to develop self-reported histories of STD, including questions about symptomatology indicative of STD. The third section gathered information about sexual behaviour, including age at first sexual relation, number of clients per week, frequency of condom use with clients and length of time working as a prostitute.

A crowding index with three categories was constructed by dividing the number of individuals per household by the number of rooms used as bedrooms. A participant's household was

defined as 'not crowded' if they scored an index of 0.25-1.5. Women living in households with crowding indices 1.51-3.5 were defined as 'semi-crowded' and participants living in households with indices >3.5 were defined as 'crowded'.

A socioeconomic index was constructed with the following indicators: education, possessions and crowding index. Each indicator was made up of three categories. For example, education was divided into 'high' for study participants who had completed technical school, high school or a professional degree; 'middle' for people with junior high school and elementary school education; and 'low' for those with incomplete elementary education or no school attendance. The construction of this socioeconomic index was meant to look for differences when the population showed homogeneous behaviour.²²

Type-specific HSV serology

The antigens used in this assay are produced in Sf-9 insect cells infected with a recombinant baculovirus expressing either the gG-1 or gG-2 gene.²³ It was developed at the CDC and the protocol as it was used for these studies has been described elsewhere.²⁴

Syphilis serology

Markers for this disease were determined, first with a non-treponemal flocculation test (RPR, Bigaux Diagnostica, S.A., Mexico City, Mexico). Second, presumptive positive results were confirmed with a specific immunofluorescence test for detecting antibodies to *Treponema pallidum* (FTA-ABS, Diagnostics Pasteur, Marnes la Coquette, France). Unconfirmed results were considered false positive sera, only samples reactive for both tests used were recorded as positive.

Data analysis

The information obtained through the questionnaires and the results of the laboratory tests were entered into DBASE III plus for handling with SPSS (PC) and EGRET statistical software. After a bivariate analysis, multivariate models were used to control for possible confounding factors.

Results

The prevalences of HSV-1 and HSV-2 infection in this population, considering that three samples were not available for laboratory testing, were 93.9% (936/997) and 60.8% (606/997), respectively. Participants seropositive for HSV-1 alone were 37.8%, and 4.6% were singly positive for HSV-2. More than half of the participants (56.2%) had been infected with both viruses, and only 1.8% of the women enrolled were seronegative for both types. The proportion of individuals with primary genital infection by HSV-1 was estimated at between 0.46% and 4.6%, according to Nahamias *et al.*¹⁶

Bivariate analyses of demographic and socioeconomic factors, history of STD exposure, and high-risk sexual behaviour in relation to HSV-1 infection are shown in Table 1. Evidence for HSV-1 infection had uniformly high prevalence values (90% even among the youngest group analysed, 16-22 years). No association was observed between the presence of HSV-1 and sexual behaviour; likewise, no association was observed with socioeconomic level. The only variable associated with seropositivity for HSV-1 was crowding, with a crude odds ratio (OR)

Table 1 Demographic, socioeconomic and sexual behaviour characteristics and history of sexually transmitted diseases (STD) according to HSV-1 prevalence in 997 prostitutes in Mexico City

Characteristics	N	Prevalence	OR ^b	95% CI
Age (years)				
16–22	302	92.72	1.00	
23–27	330	93.05	1.05	0.66–1.84
28–32	187	94.65	1.39	0.61–3.48
33–37	101	94.06	1.24	0.39–3.11
>37	77	100.00	NC ^c	–
Education				
High school/ Professional	146	92.47	1.00	
Complete elementary/ Junior high school	536	94.60	1.43	0.62–3.57
Literate/incomplete elementary school	311	93.57	1.19	0.52–2.96
Socioeconomic level				
High	168	91.67	1.00	
Middle	216	93.98	1.42	0.62–3.55
Low	397	94.71	1.63	0.71–4.07
Work site				
Bar	354	95.20	1.00	
Street	521	92.72	0.64	0.40–1.12
Place of birth				
Mexico City	263	94.30	1.00	
Mexico State	85	94.12	0.97	0.30–3.39
Other States	642	93.62	0.89	0.56–1.55
Crowding				
Not crowded	374	91.71	1.00	
Semi-crowded	486	94.44	1.54	0.96–2.69
Crowded	137	97.81	4.04	1.26–20.18
Time working in commercial sex (years)				
<1	216	92.13	1.00	
1–5	534	92.90	1.12	0.70–1.96
6–10	148	98.65	6.24	1.36–43.65
11–15	55	92.73	1.09	0.34–3.81
>15	33	100.00	NC	–
History of STD				
Gonorrhoea				
No	867	93.89	1.00	
Yes	130	93.85	0.99	0.43–2.48
Syphilis				
No	974	94.05	1.00	
Yes	23	86.96	0.43	0.13–2.11
Herpes 2^d				
No	391	96.16	1.00	
Yes	606	92.41	0.49	0.29–0.89

^a This may vary due to missing data.

^b Crude odds ratio.

^c Not calculated.

^d Presence of antibodies for HSV-2.

of 4 (95% CI : 1.3–20) for those with more than three people per bedroom. Although Table 1 also shows that working in commercial sex for 6–10 years was associated with HSV-1 seropositivity, this was not interpreted as a real association. First,

because the result is based on only two negative HSV-1 sera from 148 tested in the category and second no increasing trend was observed with increasing time in prostitution.

Among those who were seronegative for HSV-1, 75% were seropositive for HSV-2 (46/61); of the women who were seropositive for HSV-1, 60% (560/936) were reactive for HSV-2. This represents a significant difference ($P = 0.016$) and may suggest a protective effect for acquiring HSV-2 in women already infected with HSV-1. In relation to this finding, the adjusted value for HSV-1 as a risk factor for HSV-2, taking into account age, time in prostitution, place of birth and working site was shown to actually have a protective effect (OR = 0.37, 95% CI : 0.19–0.73).

The crude statistical analysis did not reveal any significant differences in risk of HSV-2 infection related to socioeconomic level, condom use, a higher number of clients or younger age at first sexual experience. Variables associated with an increased risk of HSV-2 infection are shown in Table 2, including the crude and adjusted OR in relation to age, education, type of work site, place of birth, history of STD and years working in prostitution. In Table 2 OR magnitudes after the multivariate analysis reflect the true association of variables with HSV-2 seropositivity. This is due to the control of possible confounding effects such as socioeconomic level, age, place of birth or history of STD.

As expected, a significant increase for the risk of acquiring HSV-2 infection was observed for women who were older. Women with a low level of education were significantly more likely to be infected with HSV-2, compared with women who had more education ($P = 0.046$), although the strength of this association was somewhat weak.

The type of work establishment was also associated with the risk of infection with HSV-2. Women who reported working at street sites had a 1.5 times higher risk of HSV-2 infection compared with women who worked in bars ($P = 0.012$). Women who were born outside of Mexico City and its metropolitan area also had a significantly greater risk of infection ($P = 0.001$) than people from elsewhere in the country. In this regard, Table 3 shows a bivariate analysis, demonstrating that birthplace was associated significantly with educational level and working site.

The risk for HSV-2 infection increased uniformly with the length of time actively working as a prostitute. Women with ≥ 2 years of experience were significantly more likely to be infected than women with only one year in prostitution. In general, history of STD was not predictive of infection with HSV-2; the only significant associations were observed in the crude analysis among participants who mentioned having had gonorrhoea (OR = 1.7, 95% CI : 1.1–3.0) and those individuals who tested positive for exposure to *T. pallidum* (OR = 2.8, 95% CI : 1.7–5.0). However, these associations were lost in the multivariate analysis after controlling for age, education, type of work site, place of birth, history of syphilis or gonorrhoea and years working as a prostitute.

Discussion

Biological and social factors were identified that influence the prevalence of HSV-2 among prostitutes studied in Mexico City. The biological factors were age and time working in prostitution, which are related to prolonged periods of exposure to HSV-2. These factors are linked, since older women are more

Table 2 Demographic, socioeconomic and sexual behaviour characteristics and history of sexually transmitted disease (STD) according to HSV-2 prevalence in 997 prostitutes in Mexico City

Characteristics	N ^a	Prevalence	OR ^b	Odds ratio (95% CI) ^c	P-value
Age (years)					
16–22	302	45.4	1.0	1.0	
23–27	330	64.2	2.2	1.9 (1.3–2.8)	<0.001
28–32	187	66.3	2.4	2.4 (1.4–3.8)	<0.001
33–37	101	69.3	2.7	2.7 (1.5–4.9)	<0.001
>37	77	81.8	5.4	4.1 (1.9–8.8)	<0.001
Education					
High school/Professional	146	45.8	1.0	1.0	
Complete elementary/Junior high school	536	58.2	1.6	1.5 (0.9–2.3)	0.068
Illiterate/incomplete elementary school	311	72.0	3.0	1.5 (1.0–2.7)	0.046
Work site					
Bar	354	51.6	1.0	1.0	
Street	521	66.4	1.8	1.5 (1.1–2.2)	0.012
Place of birth					
Mexico City	263	49.4	1.0	1.0	
Mexico State	85	62.3	1.6	1.9 (1.0–3.5)	0.035
Other States	642	65.2	1.9	1.8 (1.3–2.6)	<0.001
History of STD					
Gonorrhoea					
No	867	59.2	1.0	1.0	
Yes	130	71.5	1.7	1.1 (0.6–1.8)	0.771
Syphilis^d					
No	920	59.1	1.0	1.0	
Yes	77	80.5	2.8	1.9 (0.9–4.2)	0.072
Time working in commercial sex (years)					
<1	216	39.4	1.0	1.0	
1–5	534	61.6	2.5	1.9 (1.3–2.7)	<0.001
6–10	148	75.6	4.8	3.3 (1.8–5.8)	<0.001
11–15	55	80.0	6.2	3.5 (1.5–8.1)	0.002
>15	33	87.8	11.2	5.1 (1.3–19.7)	0.017

^a This may vary due to missing data.

^b Crude odds ratio.

^c Adjusted odds ratio.

^d Presence of antibodies for *T. pallidum* (reactive to both RPR/FTA-ABS).

Table 3 Relationship between prostitute's place of birth and education and working site

Characteristics	Place of birth			P-value ^a
	Mexico City n (%)	Mexico State n (%)	Other States n (%)	
Education				
High school/Professional	60 (22.8)	7 (8.2)	78 (12.2)	
Complete elementary/Junior high school	161 (61.2)	48 (56.5)	322 (50.5)	
Illiterate/incomplete elementary school	42 (16.0)	30 (35.3)	238 (37.3)	<0.001
Working site				
Bar	155 (63.3)	21 (28.8)	175 (31.7)	
Street	90 (36.7)	52 (71.2)	377 (68.3)	<0.001

^a χ^2 test.

likely to have worked longer as prostitutes. In terms of socioeconomic characteristics, women with a lower education, those who work in street sites, and those born outside Mexico City are at greatest risk of infection with HSV-2. In fact, our findings

support the hypothesis that subjects born outside Mexico City may have higher rates of HSV-2 due to socioeconomic factors, such as having a limited education and being a 'street walker' (Table 3). The particular difference seen for HSV-2 seroprevalence

between street and bar girls has been also documented by our group in a more recent study.²⁵ Additionally, a lower income, less education and longer time as a sexual worker have been documented as risk factors for HSV-2 infection among street girls from Mexico City (unpublished results).

Among the limitations of this study there is the lack of testing for reliability about past history of STD and sexual behaviour as reported by the participants. It might be important to realize for instance, that 31% of the subjects had incomplete elementary school education and that only 1.1% of them mentioned genital ulcerations. These women may also have used condoms inconsistently (although 88% reported its use), besides, the cross-sectional design of the study may have not been accurate for evaluating condom effect in a population with a relatively high HSV-2 prevalence and who were probably infected long before.

The usefulness of HSV-2 serosurveys conducted with type-specific diagnostic tests such as the Western blot assay for identifying population groups at risks for acquiring STD has been demonstrated in a number of studies.^{14,16,26} In the case of Mexico, this was the first survey on HSV-2 infection assessing a high-risk Mexican population for evidence of infection by STD.

The high seroprevalence of HSV-1 found in these women was consistent with studies conducted in other populations, for example, Japanese adults²⁷ or people from Spain,¹ and factors associated with the presence of this virus are those commonly encountered in groups of people from developing countries¹⁶ and also ethnic groups like Navajo children.²⁸ However, it should be realized that although in Mexico the variable 'crowding' is an indicator of a socioeconomic level that may have not changed significantly through the lives of these women, it is quite feasible that most HSV-1 infections occurred in childhood. Thus, current household crowding (as an adult prostitute in Mexico City) although statistically associated, is not necessarily a cause of infection.

The observation in this study that seropositive HSV-1 subjects appear to have a lower risk for acquiring HSV-2 infection, suggests that cross-protective immunity to prior HSV-1 may have protective effects that diminish the risk for infection by HSV-2. This is consistent with observations made in other studies.^{14,16}

The prevalence of HSV-2 infection was somewhat lower in this study population than that reported in female prostitute populations in the US, Africa¹⁶ or Japan.²⁷ Nonetheless, this survey identifies a core group which should receive properly designed intervention measures. This is particularly important to prevent the spread of HIV/AIDS, the seroprevalence of which has remained under 1% in Mexico City prostitutes since 1990,^{21,25,29} and CONASIDA 1996 annual report

Since HIV-1 and HSV-2 are transmitted in a similar fashion, infection patterns of HSV-2, which has a long-established stable pattern of prevalence, may be regarded as predictive for the future course of HIV-1 prevalence. Consequently, genital ulcerative disease due to viral reactivation or primary infection in these women should be prevented or reduced. Those risk factors which our study found to be significant for acquiring HSV-2 should be taken into account in developing educational materials promoting safe sexual behaviours and the use of protective barriers to maintain a low rate of HIV infection in this population. Regarding the issue of condom use, even though we did not see a protective effect for HSV-2 acquisition among women studied (crude OR = 0.9, 95% CI : 0.2–4.8), the need remains to perform

further research to address the matter. So far, there is only one HSV-2 study (in Costa Rican women) that has showed a small significant effect for condom use conferring protection from genital herpes.³⁰

Finally, to continue the description of HSV-2 epidemiology in Mexico, we are extending our research (including the use of viral culture in some groups), and assessing the seroincidence of the viral infection in prostitutes, as well as the seroprevalence and risk factors of herpes among low-risk women, heterosexual and homosexual men.^{29,31–33} Some data from these studies are, for example, a 4.2% HSV-2 prevalence by tissue culture among women in a cancer detection programme in the City of Cuernavaca;³¹ HSV-2 seroprevalences observed in Mexico City of 17.9% in women seeking family planning services; 29.3% in non-prostitute females attending an AIDS/STD counselling facility;³³ 32.4% in heterosexual male employees of bars where female prostitution takes place³² and as high as 51.4% in male homosexuals in comparison to 14.6% in heterosexual men (unpublished data).

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