Prevalence of Herpes Simplex Type 2 Antibodies and a Clinical History of Herpes in Three Different Populations in Campinas City, Brazil

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ABSTRACT

Objectives: To determine the seroprevalence of herpes simplex virus type 2 (HSV-2) antibodies and the relation between the history of clinical herpes and the presence of type-specific HSV-2 antibodies in three different populations from the city of Campinas City, Brazil.

Population and methods: One hundred and one college students, 96 patients with sexually transmitted diseases (STD), and 102 women at delivery were interviewed and blood samples were collected. Total HSV (HSV-1 and HSV-2) antibodies were screened by enzyme-linked immunosorbent assay (ELISA) and type-specific HSV-2 antibodies were detected by Western blot assay.

Results: Herpes simplex virus antibodies were detected in 66.3% of the students, 97.1% of the women at delivery, and 99.0% of the STD patients. Type-specific HSV-2 antibodies were detected in 6.9% of the students, 22.6% of the women at delivery, and in 53.1% of the STD patients. History of genital herpes was reported by none of the students, by one of the women at delivery, and by 11 of 51 (21.6%) STD patients who were HSV-2 seropositive. Four of the 45 (8.9%) seronegative STD patients reported a history of genital herpes.

Conclusion: The prevalence of HSV-2 infection in Campinas City can be significantly affected by the characteristics of the population studied, as was shown in previous studies. The sensitivity of the history of genital herpes was low in the present series, stressing that prophylactic measures for vertical and horizontal transmission of HSV-2 should not be based only on a positive history of genital ulcers.

Key Words: *herpes simplex virus 2, seroepidemiology, Western blot*

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Herpes simplex virus (HSV) type 2 is the principal cause of genital herpes, a life-long infection that may result in frequent and severe recurrent genital lesions accompanied by serious emotional and psychological problems.[1-3] Unrecognized symptomatic cases and frequent asymptomatic viral shedding are the main source of continued genital HSV-2 transmission.[4,5] The asymptomatic shedding in the genital tract at the time of delivery is responsible for most neonatal HSV-2 infection.[6-8] The acquisition of genital herpes during pregnancy has been associated with spontaneous abortion, prematurity, and serious consequences in neonates born to infected women, including severe neurodevelopmental disabilities and death.[9-12] In addition, HSV-2 genital herpes has been considered an important risk factor for the acquisition of human immunodeficiency virus (HIV) infection.[13]

The introduction of type-specific tests for detection of HSV-2 antibodies allowed accurate seroepidemiologic studies of the HSV-2 infection.[14-16] The prevalence has been correlated with demographic and epidemiologic factors such as age, sex, race, ethnic group, lifetime number of sexual partners, age at first sexual intercourse, and previous history of sexually transmitted diseases.[17-20] Despite the availability of diagnostic techniques and antiviral therapy, the incidence of HSV-2 infection is increasing in the world, which indicates the need of control measures.[21]

The objective of the present study was to evaluate the prevalence of type-specific HSV-2 antibodies and the relation of HSV-2 antibodies with the history of genital herpes in three different populations in Campinas City, Brazil.

MATERIAL AND METHODS

Study Population

The study was conducted at the University of Campinas (UNICAMP), in Campinas City, Brazil, from 1993 to 1997. Campinas City is an industrialized city with 908,000 inhabitants, with a mean income of US $9800 per year, located in the State of São Paulo.

The study was approved by the local Research and Ethics Committee. After informed consent had been signed, the participants were interviewed and had blood samples collected. All participants were asked about the degree of education, number of sexual partners in the previous year, and history of clinically diagnosed genital herpes. The following groups were enrolled in the study:

1. 96 low-income patients, aged 14 to 57 years, 44 female and 52 male, attending a public sexually transmitted diseases (STD) clinic.
2. 102 women at delivery, 14 to 42 years of age, at the University Hospital, which serves a low income population.
3. 101 college students, 17 to 30 years of age, 41 female and 60 male, from UNICAMP.

Serologic Tests

Herpes simplex virus (HSV-1/HSV-2) antibodies were screened by an in-house enzyme-linked immunosorbent assay (ELISA) with viral antigens from pooled Vero cells infected with HSV-1 (McIntyre strain) and HSV-2 (Johnson N strain) that were solubilized by sonication in phosphate buffered saline (PBS), pH 7.2, containing 0.2% sodium deoxycholate. Control antigen was prepared with uninfected Vero cells. Microtiter plates (Polysorp, Nalge Nunc International Corporation, Rochester, NY) coated with HSV-1 and HSV-2 antigens were blocked with PBS containing 5% nonfat dry milk, 0.01% thimerosal, and 0.1% Tween 80 (buffered saline), and a 1:100 serum dilution was dispensed into wells coated with either virus or control antigen, and incubated for 1 hour at 37°C. After four washes with PBS containing 0.1% Tween 80 (PBST), peroxidase-conjugated antihuman IgG (Gibco BRL) was added, and the plates were incubated for 40 minutes at 37°C. After four additional washes, chromogenic substrate containing 0.02% hydrogen peroxide and 0.05% o-phenylenediamine in 0.05 M sodium citrate buffer, pH 5.0, was added. The enzymatic reaction was stopped with 2.5 N H_2SO_4 and absorbance was read at 492 nm. The results for each serum sample, expressed as ΔOD (difference of optical density), were obtained by subtracting the absorbance of the control antigen from the absorbance of HSV antigen, and sera presenting ΔOD 0.3 or higher were considered positive.

Herpes simplex virus-2 type-specific antibodies were detected in ELISA-positive samples by Western blot analyses as described elsewhere.[16] Antigen was prepared with Vero cells infected with HSV-2 (Johnson N strain) solubilized with lysis buffer (10 mM Tris-HCl, pH 7.5, 1% Nonidet® -P40, PMSF 1 mM). The HSV-2 antigen with equal volume of sample buffer (10 mM Tris, pH 6.8, 1 mM EDTA, 8% sodium dodecyl sulfate, 20% glycerol, 5% 2-mercaptoethanol, and 0.01% bromophenol blue) was boiled for 5 minutes, followed by electrophoresis in 8% discontinuous polyacrylamide gels with mini-Protean II apparatus (Bio-Rad Laboratories, Richmond, CA), and then transferred to nitrocellulose at 70 mA for 1 hour. The nitrocellulose was then cut in strips and blocked for 2 hours with BS and incubated overnight at room temperature with 1 mL of serum sample diluted 1:50 in PBS. Nitrocellulose strips were washed four times for 5 minutes each with PBST. After incubation with peroxidase-conjugated antihuman IgG (Gibco BRL) for 1 hour, the strips were washed four times with PBST, rinsed once with Tris-buffered saline (500 mM NaCl in 20 mM Tris, pH 7.5) (TBS), and stained for 10 minutes with chromogenic substrate (30 mg of 4-cloro-1-naphthol in 10 mL of cold methanol, 30 μL of 30% H_2O_2 and 50 mL of TBS). The strips were then washed with water and allowed to dry. Seropositivity for HSV-2 was defined by the presence of the 92,000-M_r band of gG-2.

Statistical Analyses

Chi-square and Fisher's exact tests for proportions and chi-square for linear trend were applied for comparison of prevalence rates, using an EPI-6 software.

RESULTS

Sixty-seven of the 101 (66.3%) students had total HSV antibodies, as detected by ELISA. This prevalence was low ($P < 0.0001$) compared with the group of women at delivery (97.1%) and STD patients (99.0%). The prevalence of total HSV antibodies was similar in all age groups in the three populations.

The overall prevalence of type-specific HSV-2 antibodies was 6.9% in the student group, 22.6% in the women at delivery, and 53.1% in the STD patients ($P < 0.0001$). Differing from total HSV antibodies, the prevalence of type-specific HSV-2 antibodies increased with age in the group of STD patients ($P < 0.05$). In the women at delivery the prevalence rates increased in the different age groups up to 30 years ($P < 0.01$). Unexpectedly, after 30 years of age the prevalence rate decreased (Table 1).

Among the students the prevalence rates of HSV-2 antibodies were similar in males (6.7%) and females (7.3%), but in STD patients the rate was significantly higher ($P < 0.05$) in males (63.5%) than in females (40.9%). Thirty-two (61.5%) of the males reported having more than one sexual partner in the previous year compared with nine (20.5%) of female patients ($P < 0.0001$).

When only individuals who had HSV-2 antibodies were considered, a history of genital herpes was reported by none of the seven students, by 1 of 23 (4.3%) of the women at delivery, and by 11 of 51 (21.6%) of the STD patients. Seven of the 11 STD patients who had a history of genital herpes had ulcers clinically diagnosed as genital

Table 1. Prevalence of HSV and Type-Specific HSV-2 Antibodies by Age Group

	Women at Delivery		*College Students*		*STD Patients*	
Age (y)	*HSV**	*HSV-2†*	*HSV**	*HSV-2†*	*HSV**	*HSV-2†*
≤ 20	33 (94.3)	4 (11.4)	28 (70.0)	2 (5.0)	17 (94.4)	7 (38.9)
21–25	20 (95.2)	5 (23.8)	36 (64.3)	5 (9.8)	26 (100.0)	11 (42.3)
26–30	19 (100.0)	9 (47.4)	3 (60.0)	0 (0.0)	12 (100.0)	7 (58.3)
>30	27 (100.0)	5 (18.5)	–	–	40 (100.0)	26 (65.0)
Total	99 (97.1)	23 (22.5)	67 (66.3)	7 (6.9)	95 (99.0)	51 (53.1)

*ELISA; †Western blot. Note: Data are n° positive (%).

HSV infection when admitted in the study. Among the HSV-2-negative patients, 4 of 45 (8.9%) reported a history of genital herpes. The sensitivity of the history of genital herpes in STD patients was 21.6% and the specificity was 91.1% (Table 2).

In addition, four HSV-2-negative patients had a clinical diagnosis of genital herpes when admitted to the study but gave no history of the disease; all four had total HSV antibodies detected by ELISA.

DISCUSSION

In the present study, the prevalence of HSV infection was similar in all age groups in the three populations, a finding which was somewhat expected in an almost exclusively adult study group. The two groups with a lower level of education (i.e., women at delivery and STD patients) had higher rates of HSV antibodies (97.1% and 99.0%, respectively) than the college students (66.3%). In Brazil, education level correlates with socioeconomic status and almost all college students belong to a high socioeconomic level. These results are similar to those of studies in adult populations from developed countries, where HSV antibodies have been detected in 30 to 50% of high and 80 to 100% of low socioeconomic level groups.[1]

In the present study, the prevalence of type-specific HSV-2 antibodies increased with age. However, no explanation was found for the unexpected lower prevalence rate of HSV-2 antibodies after 30 years of age in the group of women at delivery. In this group, the overall prevalence (22.5%) was lower than that seen in a previous study, conducted in São Paulo in 1988, which reported 42% and 31% prevalence rates, in the low and intermediate socioeconomic classes, respectively.[22] Western blot assay was used in both studies to detect HSV-2 antibodies. The lower prevalence rate found in the Campinas City study might be attributed to differences in socioeconomic status and sexual behavior between the two populations. Campinas is a city with a higher *per capita* income, and is about 15 times smaller than São Paulo. Changes in sexual behavior after the epidemic of acquired immunodeficiency syndrome (AIDS) also might have played a role in the incidence of HSV-2 infections. The prevalence of HSV-2 antibodies in this group was similar to that found in studies conducted in a matched group in Europe and in the USA.[23-25] The low prevalence of HSV-2 infection observed among college students (6.9%), was similar to that reported in American students.[26,27]

Table 2. History of Genital Herpes and Seropositivity by Western Blot Assay in the Group of STD Patients

	Western Blot	
History of Genital Herpes n = 96 (100%)	*Positive n = 51 (100%)*	*Negative n = 45 (100%)*
Positive 15 (15.6)	11 (21.6)	4 (8.9)
Negative 81 (84.4)	40 (78.4)	41 (91.1)

Among the STD patients (all HIV-negative), 53.1% had HSV-2 type-specific antibodies. This prevalence is low compared to a study conducted in Rio de Janeiro, Brazil, by Rosa-Santos et al,[28] where 72% of the patients had HSV-2 antibodies measured by type-specific enzyme immunoassay (EIA). As it has been demonstrated that the type-specific EIA and the Western blot assay have similar sensitivities,[16] the authors believe that the difference in HSV-2 prevalence found in these two populations could be explained by the inclusion of HIV-positive patients in the study in Rio de Janeiro. Gwanzura et al reported HSV-2 seroprevalence of 35.7% among HIV-negative subjects and 82.7% among HIV-positive subjects in Zimbabwe.[29] Although the overall prevalence of HSV-2 antibodies in the group of STD patients was similar to that reported in STD clinics of other countries,[25,30] the higher seroprevalence in males was unexpected. This finding might be explained by a difference in sexual behavior, as a higher number of sexual partners was reported in the male group than among the females.

The sensitivity of the history of genital herpes was low in the group of women at delivery and the students. Only one of the 23 HSV-2-seropositive women and none of the students had a history of genital herpes. A low sensitivity (<10%) of the history of genital herpes also was reported by Fleming et al in their study of a noninstitutionalized population of Americans.[18] Moreover, only 11 of 51 (21.6%) STD patients with antibodies to HSV-2 gave a history of genital herpes. These results are similar to findings reported in Australia, where the HSV-2 sero-

prevalence in STD patients was 64%, but only 24% reported a history of genital herpes.[31]

The higher sensitivity of the history of genital herpes among the HSV-2-positive STD patients, compared to women at delivery and students, can be attributed to their awareness of the infection, since 7 of 11 patients who had history of genital herpes were attending the STD clinic for the same reason at the time of sample collection.

A clinical history of genital herpes also was reported by 4 of 45 (8.9%) HSV-2-seronegative STD patients. This result suggests that genital herpes in these patients was caused by HSV-1, as they were all ELISA positive. A wide range (7-50%) of proportion of isolates of HSV-1 from first episodes of genital herpes has been reported in various countries,[32] but in Brazil information concerning the type of HSV associated with genital herpes is not available. However, a misdiagnosis of genital herpes also could have occurred, since HSV culture or detection of HSV antigens was not performed to confirm the clinical diagnosis of genital herpes. Additionally, a failure in antibody testing also must be considered.

The four HSV-2-negative patients with genital ulcer at the time of sample collection had no history of genital herpes. This finding could be due to a primary HSV-2 genital infection, as it has been demonstrated that seroconversion is detected by Western blot assay in only 25% of samples drawn earlier than 21 days from the onset of the disease.[16] In these cases an incorrect clinical diagnosis, HSV-1 infection, or a failure in antibody testing also must be considered.

CONCLUSION

In Campinas City, the prevalence of HSV-2 infection varies significantly according to the characteristics of the population studied. The sensitivity of the history of genital herpes is low, indicating that prophylactic measures for vertical and horizontal transmission of HSV-2 should not be based only on a positive history of genital ulcers.

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