

HERPES SIMPLEX VIRUS TYPE 2 INFECTION IN PREGNANCY: ASYMPTOMATIC VIRAL EXCRETION AT DELIVERY AND SEROEPIDEMIOLOGIC SURVEY OF TWO SOCIOECONOMICALLY DISTINCT POPULATIONS IN SÃO PAULO, BRAZIL

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SUMMARY

The objective of the present study was to estimate the prevalence of herpes simplex virus type 2 (HSV 2) antibodies in child bearing women of 2 Brazilian populations with different socioeconomic status and to determine the risk of neonatal HSV exposure by means of maternal cultures at the onset of labor. The study was conducted at 2 hospitals: A, serving very low income patients and B, serving middle socioeconomic class. 173 participants from group A and 127 from B answered a questionnaire which showed that the patients had similar ages (27.7 and 26.8 years, respectively) but differed with regard to socioeconomic status, age at first intercourse (18.6 vs 20.6 years), number of sex partners (1.5 vs 1.2) and previous sexually transmitted diseases (15% vs. 1.5%). History of genital herpes was given by 11% of group A participants and by a similar number, 7%, of patients from group B. In addition, 200 serum samples from population A and 455 from B were tested by ELISA for anti HSV antibodies and 92% and 86%, respectively, were found to be positive. Sixty seropositive samples from group A and 90 from B were further analyzed by Western blot, which showed the presence of type 2 specific antibodies in 46% and 36%, respectively, suggesting an overall HSV 2 prevalence of 42% in group A and 31% in B. Cervical specimens were obtained for culture from 299 asymptomatic patients of population A and 313 of B. HSV was isolated from one specimen in each group, indicating a 0.3% incidence of asymptomatic viral excretion in both populations. In conclusion, the prevalence of type 2 antibodies in childbearing women was very high, but it did not differ with the socioeconomic status. The risk of HSV perinatal transmission was also similar in the 2 study populations and it was comparable with the data from developed countries. Our findings do not indicate the need of special screening programs for asymptomatic HSV excretion in Brazilian pregnant women.

KEY WORDS: Herpes simplex; Genital herpes; Pregnancy; Neonatal herpes.

INTRODUCTION

Herpes simplex virus type 2 (HSV 2) most commonly determines sexually transmitted genital infections. Recent surveys have shown an increasing prevalence of genital herpes in the adult population of developed countries¹. Infection in neonates, although comparably less

frequent¹⁰, is a serious disease associated with a high morbidity and mortality even with antiviral therapies¹⁴. Since the majority of the infants acquires HSV during passage through a contaminated birth canal, exposure can be prevented by cesarean delivery if maternal herpes

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is recognized at the onset of labor. However, antepartum cervical cultures could not predict the perinatal transmission risk of HSV in women with recurrent genital herpes¹. In addition, most neonates who develop HSV disease are born to mothers without previous history of genital herpes^{3,8}. At the present time, the exposure of newborn babies to an asymptomatic maternal HSV infection at delivery is neither predictable nor preventable.

In Brazil, the impact of HSV 2 infections in the general population or among neonates has not yet been studied because of the restricted use of viral culture. Furthermore, the limitations in specificity of serologic assays for type 2 antibodies hindered the development of epidemiologic surveys. Recently, type specific antibody measurement techniques have been established^{2,6}, allowing the development of seroepidemiologic studies.

The goal of the present study was to estimate the prevalence of HSV 2 antibodies in child bearing women in 2 populations with different socioeconomic status and to determine the risk of neonatal HSV exposure by means of maternal cultures at the onset of labor. Since the prevalence of HSV 2 infections was associated with specific demographic characteristics^{4,9}, the data from underdeveloped countries could theoretically be different from the highly industrialized world and could also vary among populations with distinct socioeconomic status.

MATERIAL AND METHODS

Study Design

Two distinct populations, A and B, were evaluated in this study. Group A consisted of women who arrived in labor between March 1988 and May 1989 at the Hospital das Clínicas of the University of São Paulo Medical School, which serves a very low income population. Group B was represented by women in labor who were admitted during the same period of time at the Hospital do Servidor Público Estadual, which serves mostly low and middle class. All the women who consented to enter the study were eligible, except those who had clinical evidence of active genital herpes at the time of delivery. Specimens for viral culture were taken before delivery. The participants were later interviewed by one of the investigators with regard to demographic characteristics

(age, marital status, education, occupation, husband's education and occupation, number of children, living conditions), previous history of herpes simplex virus infections for both patient and last sex partner and sexually transmitted diseases other than genital herpes (clinical history of syphilis, gonorrhoea, warts, vaginal discharge and syphilis serology). The socioeconomic status was determined as previously described⁷. Blood samples were obtained for antibody studies. In group A the blood samples were collected by the investigators and belonged to the women who had specimens taken for viral cultures. In group B the venipunctures were performed by the staff of the obstetrical service and represented a random sample of the women who had delivered at that institution during the study period.

Viral Culture and Identification

Specimens to be cultured for HSV were obtained from the cervix of the participants with a swab that had a cotton tip. Although asymptomatic HSV excretion from the vulva is at least equally frequent to the cervical shedding, the latter has been shown to have better correlation with neonatal infection³. The swab was placed in 1 ml of Hank's solution supplemented with penicillin, amikacin and amphotericin B and stored at 4°C before transportation to the laboratory. Each specimen was inoculated into duplicate tubes (0.2 ml per tube) containing confluent monolayers of human foreskin fibroblasts (HFF) and Vero cells. The cultures were maintained for 2 weeks in Eagle's minimal essential medium containing 2% fetal calf serum and were daily examined for cytopathic effect.

HSV was confirmed by immunoblot procedure¹³. Briefly, 10 µl of detergent solubilized extract of the test cultures was blotted onto nitrocellulose filters and allowed to dry. Unbound sites were blocked with bovine serum albumin. After washing the filters with 0.05% Tween 80 in phosphate buffered saline (PBS-T), guinea pig hyperimmune anti-HSV 2 antiserum was added overnight at 4°C. Bound antibodies were revealed with goat anti-guinea pig IgG antiserum conjugated to horseradish peroxidase (Sigma, St. Louis, Mo) and diaminobenzidine (Sigma). Uninfected fibroblasts, stock herpes simplex virus varicella zoster virus and cytomegalovirus were used as controls.

Antibody Determinations

Antibodies to herpes simplex virus were screened by ELISA, as previously described¹¹. All sera were tested at 1:30 dilution against a solubilized extract of Vero cells infected with HSV 1 strain Mc Intyre. Sixty seropositive samples from group A and 94 from group B were randomly selected with the use of specific tables for further Western blot¹² testing for anti-HSV 2 specific antibodies. Briefly, detergent solubilized extracts of human foreskin fibroblasts infected with HSV 1 strain McIntyre and HSV 2 strain MS were electrophoresed in 7% acrylamide 4% sodium dodecylsulfate gels. The polypeptides were electrophoretically transferred to nitrocellulose using the method described by Towbin. The blots were cut into strips containing both HSV 1 and HSV 2 antigens, washed with PBS-T, blocked with 10% skimmed milk in PBS, and incubated with 1/20 dilutions of the test sera in PBS-T. Specific binding was detected using goat anti-human IgG antiserum conjugated with horseradish peroxidase (Sigma) and diaminobenzidine. Human and guinea pig hyperimmune sera of known specificity were used to establish the optimal antigen concentration that allowed a qualitative assessment of the presence of antibodies directed against HSV 2 type specific polypeptides. Figure 1 shows typical representations of the Western blot results in our population.

RESULTS

Between March 1988 and May 1989, 612 women were recruited for the study: 299 delivered at the Hospital das Clínicas of the University of São Paulo Medical School, group A, and 313 were admitted at the Hospital do Servidor Público Estadual, group B. Of all participants, 173 from population A and 127 from group B answered the study questionnaire. The two groups were similar with regard to age, but there was a significant difference in their socioeconomic status assessed by the level of education and occupation of the patient and husband. Patients in group A (low income population) began their sexually active life significantly earlier than in group B, there was a higher mean of previous sex partners in group A as compared with B, and more patients with previous sexually transmitted diseases (table 1). No significant differences were noted with regard

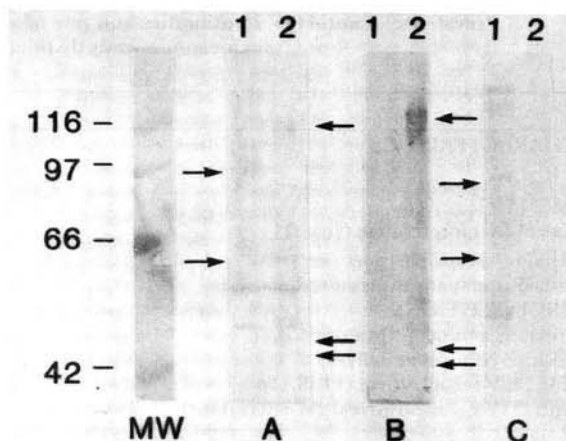


Fig.1 - Strips represent typical Western blot analysis derived from 155 HSV seropositive patients. Arrows indicate type specific polypeptides. MW = molecular weights, 1 = HSV 1, 2 = HSV 2. A: sample positive for both HSV 1 and 2; B: HSV 1 negative, HSV 2 positive; C: HSV 1 positive HSV 2 negative.

to previous genital herpes: 11% and 7% in group A and B, respectively. In addition, the status of the last sex partner with regard to herpes simplex infection was unknown by most of the patients.

Among 200 group A patients from whom blood samples were obtained, 183 (92%) had antibodies anti-HSV measured by ELISA. Sixty randomly selected sera were further tested by Western blot for specific anti-HSV 2 immunity and 28 (46%) showed type 2 antibodies implying an overall type 2 antibody prevalence of 42%. Similar results were found in 455 blood samples derived from patients who delivered at the group B hospital: 391 (86%) displayed HSV humoral immunity and 34 out of 94 (36%) had type 2 antibodies with an overall prevalence of 31%.

During the study, 299 cervical specimens were obtained for culture from group A, and 313 from population B. HSV was isolated from one specimen in each group, determining a 0.3% incidence of asymptomatic cervical shedding in both populations. The viruses were detected after 5 and 3 days, respectively, of culture. One of the 2 infants vaginally delivered through a contaminated genital tract died of respiratory failure 24 hs after birth. The respiratory distress was clinically ascribed to hialine membrane, without any obvious causes. However, necropsy or other invasive diagnostic studies were not performed, no specimens were

TABLE I
Prevalence of anti-HSV 2 antibodies and rate of asymptomatic cervical HSV excretion at delivery in two socioeconomically distinct populations, São Paulo, Brazil.

CHARACTERISTICS	Group A	Group B	p value
	n = 173	n = 127	
Age (years)	27.7±.7	26.8±5.8	.28 ^b
Age at first intercourse (years)	18.6±3.8	20.6±4.3	.0001 ^b
Total n ^a of sex partners	1.5±.62	1.2±.56	.004 ^b
Previous sexually transmitted diseases	26/173(15%)	2/127(1.5%)	.0001 ^c
HSV INFECTIONS			
Previous genital herpes	20/173(11%)	9/127(7%)	.19 ^c
Positive cultures at delivery	1/299(.33%)	1/313(.31%)	.97 ^c
Anti-HSV seropositives (ELISA)	183/200(92%)	391/455(86%)	.61 ^c
Anti-HSV 2 seropositives ^d (Western blot)	28/60(46%)	34/94(36%)	.19 ^c

a. Numbers represent mean±S.D.

b. Student's T statistics

c. X² statistics

d. Anti-HSV 2 antibodies status was evaluated in anti-HSV positive serum samples.

available for viral culture from the child and maternal serology was declined. The other neonate did not present any signs of herpes simplex infection during the first 2 days of life and was lost to follow-up thereafter. The mother had anti-HSV 2 antibodies.

DISCUSSION

This study shows that HSV 2 infections are very common in child bearing women of low and middle socioeconomic class in Brazil, with an estimated antibody prevalence of 42% and 31%, respectively. The prevalence of type 2 antibodies observed in these Brazilian populations ranks among the highest described for any of the subgroups studied in the developed countries^{1,9}.

The encounter of HSV antibodies in general was also extremely frequent and ranged from 92% in group A to 86% in population B.

Although variables related to sexual behavior, such as number of sex partners, age at first intercourse and other sexually transmitted diseases significantly differed in association with the socioeconomic factors, the data did not indicate a strong correlation between the socioeconomic status and the HSV 2 distribution. This finding is in accordance with other reports where the socioeconomic factors had either a small or a nonsignificant effect on the prevalence of type 2 antibodies^{1,8,9}. Sexual behavior and race were shown elsewhere to affect the distribution

of HSV 2 antibodies, but these variables were not independently studied in this survey.

Although HSV 2 antibodies were quite frequent in the study population, clinical expression of the infection was rare. History of genital herpes was obtained from 11% of the women in group A and 7% in B, which indicates that only 20% to 25% of the patients were aware of the HSV 2 infection. This confirms that clinical information greatly underestimates the prevalence of HSV 2 disease and underscores the importance of teaching these patients how to identify the episodes of active genital herpes⁵. Furthermore, asymptomatic cervical excretion of HSV 2 was detected in only 0.3% of the women and one more patient was identified with active herpetic lesions (data not shown). Neonatal herpes simplex was also infrequent. One of the babies exposed to HSV at delivery died 24 hours after birth and the cause of death remained unknown. The other exposed infant, born during an asymptomatic HSV recurrence, did not return at the follow up visit. No other neonates were clinically suspected of herpes at the time the study was conducted in both hospitals.

There are no previous published studies addressing the incidence of HSV shedding or neonatal herpes simplex in Brazil. Even during the present study, cultural and economic difficulties determined that only half of the participating patients answered the questionnaire. Although this might have introduced a potential bias, the 2 study populations were homoge-

neous enough to allow us to use the data. In addition, only 155 Western blots could be performed during this study. In order to avoid potential biases, a randomization table was used for the selection of the sera.

In conclusion, despite the very high prevalence of type 2 antibodies in child bearing women, the risk of HSV exposure at birth and the incidence of neonatal herpes were quite low. The numbers were comparable with the data from developed countries and were not associated to the socioeconomic status. Our findings do not indicate the need of special screening programs for asymptomatic HSV excretion in Brazilian pregnant women. At present, we recommend that all pregnant women, regardless of their history of genital herpes be assessed for herpetic lesions upon admission in labor and cesarean deliveries be offered in the positive cases. Since exposure to asymptomatic excretion is unpredictable, pediatricians need to consider neonatal herpes in the differential diagnosis when infants become ill during the first weeks of life.

RESUMO

Infecções por herpes simplex tipo 2 durante a gestação: excreção assintomática do vírus durante o parto e estudo soropidemiológico de duas populações socioeconomicamente distintas.

O objetivo deste estudo constou em determinar a prevalência dos anticorpos anti vírus herpes simplex (HSV) tipo 2 em mulheres em idade fértil, pertencendo a 2 populações socioeconomicamente distintas, e verificar o risco de exposição neonatal a HSV, por meio de isolamento de vírus no início do parto. O estudo foi realizado em 2 hospitais: A, servindo uma população de baixa renda; B, servindo a classe média. 173 participantes do grupo A e 127 do grupo B responderam um questionário que mostrou que os pacientes pertenciam à mesma faixa etária (27,7 e 26,8 anos, respectivamente) mas divergiam quanto ao estado socioeconômico, idade ao início da vida sexual (18,6 e 20,6, respectivamente), número de parceiros sexuais (1,5 comparado à 1,2) e doenças sexualmente transmitidas (15% e 1,5%, respectivamente). Uma história de herpes genital no passado foi obtida em números semelhantes nos dois gru-

pos, 11% do grupo A e 7% do B. 200 soros do grupo A e 455 do B foram analisados por ELISA quanto à presença de anticorpos anti HSV, e 92% e 86%, respectivamente, foram positivos. 60 amostras soropositivas do grupo A e 90 do B foram analisados por Western blot, que mostrou anticorpos anti HSV 2 em 42% do grupo A e 31% do B. Material cervical foi obtido para isolamento de vírus de 299 parturientes assintomáticas do grupo A e 313 do B. Uma paciente em cada grupo teve isolamento positivo para HSV, indicando uma incidência de 0,3% de excreção vírica assintomática em ambos os grupos. Em conclusão, a prevalência de anticorpos anti HSV 2 em mulheres em idade fértil em São Paulo se mostrou muito elevada, mas não variou com o estado socioeconômico da população. O risco da transmissão perinatal do HSV foi semelhante nas duas populações e comparável aos números relatados em países desenvolvidos. Nossos achados não indicam a necessidade de programas especiais de detecção da excreção assintomática de HSV em gestantes do Brasil.

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