Seroepidemiological study of herpes simplex virus type 2 in patients with the acquired immunodeficiency syndrome in the City of Niterói, Rio de Janeiro, Brazil

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This study was designed to determine the seroprevalence of herpes simplex virus type 2 (HSV-2) and to evaluate its association with age, sex as well as other demographic and behavioural factors in 150 human immunodeficiency virus (HIV) positive adults patients attending the general medical outpatient ward for routine care of Niterói, state of Rio de Janeiro, Brazil. Serum samples were screened for HSV-2 antibodies using an indirect ELISA. Eighty-three patients were men (mean age: 38.8) and 67 were women (mean age: 35.4). The estimated prevalence of HSV-2 was 52% (95% CI: 44-60%) and it was higher among men (53%) than among women (50.7%). Overall, the age of first sexual intercourse and past history of genital herpes were associated with HSV-2 seropositivity. Analysis by gender disclosed significant association of number of lifetime sex partners only among men. Although HSV-2 antibodies were frequent in the study group, genital herpes was reported by 21.8% of the HSV-2 positive subjects, indicating low awareness of the HSV-2 infection. These results may have public health importance for Brazil as the high rate of HSV-2 infection may act as a cofactor of HIV transmission.

Key words: herpes simplex virus type 2 - seroprevalence - human immunodeficiency virus positive - Brazil

Infection with herpes simplex virus (HSV) is among the commonest human viral infections (Ashley et al. 1990). Typically, HSV-1 causes orolabial herpes and HSV-2 causes genital herpes, although either type of virus can cause any of those clinical syndromes (Whitley & Gnann 1993).

HSV-2 is one of the commonest causes of genital ulceration worldwide, but the relative importance of infection appears to vary from place to place. In countries where co-infection with human immunodeficiency virus (HIV) and HSV-2 is common the significance of HSV-2 as a cause of ulceration has probably been underestimated (Cowan et al. 2003). The ulceration that occurs in immunocompromised individuals is frequently atypical, severe, and persistent (McGrath & Newman 1994, Suligoi et al. 2002) and may mimic ulceration caused by other organisms, such as syphilis and chancroid (Chen et al. 2000).

There is increasing evidence that HSV-2 facilitates HIV transmission (Wasserheit 1992, Wald & Link 2002), which strengthens the importance of the implementation of available HSV control methods and the development of vaccines (Corey & Handsfield 2000, WHO & UNAIDS 2001, Cowan et al. 2003). The majority of HSV infections are unrecognized and most people who are infected shed virus at some time and are, therefore, potentially infectious. Therefore, seroepidemiological studies are critical to understanding the pattern and distribution of infection within populations (Cowan et al. 2003). Nonetheless, data on the prevalence of HSV-2 infection among HIV-positive individuals are scarce. Recently, commercially available type-specific serologic assays allowed more extensive study of the epidemiology of HSV-2 infections (Ashley et al. 1998, Cowan et al. 2003, Turner et al. 2003).

The objectives of the current study were to determine the seroprevalence of HSV-2 and its association with age, sex as well as other demographic and behavioural factors among HIV-positive patients from a large general hospital in Niterói, state of Rio de Janeiro, Brazil.

PATIENTS AND METHODS

Subjects - The study was conducted in the Hospital Universitario Antonio Pedro, Federal Fluminense University, a large tertiary public hospital located in Niterói, state of Rio de Janeiro, Brazil. Niterói has around 474,000 inhabitants and the hospital provides health care to neighboring municipalities and to a lesser extent to populations from other parts of the state. The study population consisted of all HIV-positive adult patients attending the general medical outpatient care for routine CD4 lymphocyte counts and HIV viral load estimation, between November 2001 and June 2002. Written informed consent was obtained and the project was approved by the Hospital Review Board. Patients were informed of the tests

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results and offered treatment, if necessary. Blood samples were assigned a numerical code to conceal the identity of the study subjects.

The patients were interviewed using a standardized questionnaire that included information on demographic variables (gender, age, level of education, residence), behaviour practices (number of lifetime sex partners, age of first sexual intercourse), and past history of sexually transmitted diseases (STD) and genital herpes. Serum samples were collected from each participant and linked to the questionnaire by the aforementioned numerical code.

Serological tests - Sera were screened for HVS-2 immunoglobulin G antibodies using an indirect HSV-2 enzyme-linked immunosorbent assay (HerpesSelect, Focus Technologies, Cypress, CA, US), which has an estimated sensitivity of 98% and an estimated specificity of 97% (Ashley et al. 1998). The test uses recombinant HSV-2 gG2 antigen to identify HSV-2 antibodies, and was run according to the manufacturer's specifications, with control sera in the same test plate. Results were reported according to the manufacturer's cut-off values.

Data analysis - Data were entered and analyzed using Epi Info 2002 (Centers for Diseases Control, US). Odds ratios and their 95% confidence intervals were calculated to measure the association between HSV-2 serological infection and risk factors, including demographic characteristics, behavioural factors, STD history, and CD4 cell counts. Continuous variables (e.g. age, age of coital debut, number of partners, or CD4 count) were categorized. Differences in proportions were assessed using the chi-square test and chi-square test for linear trend, when appropriate.

RESULTS

During the study period 153 HIV/AIDS patients attended the hospital for routine care and 150 (98%) agreed to participate in the study comprising 83 (55.3%) men (mean age: 38.8 years; range: 20 to 62) and 67 (44.7%) women (mean age: 35.4 years; range: 20 to 63). Thirty-nine percent (59/150) of subjects originated from São Gonçalo, 35% (53/150) from Niterói, and 22% (33/150) from other municipalities of the state of Rio de Janeiro. With regard to HIV exposure category, 88 (58.7%) were classified as heterosexuals, 39 (25.7%) were classified as men who have sex with men (MSM), 8 (5.3%) were considered to have been infected through blood transfusion, 3 (2%) were intravenous drug users (IDU), and 12 (8%) had an unknown exposure. The overall prevalence of HSV-2 was 52% (95% CI: 44 - 60%). Seroprevalence was slightly higher among men (Table). However, when MSM were excluded, seroprevalence was higher, although not significantly, among women (50.7% vs 54.5%; p = 0.5855).

Study subjects with a past history of genital herpes were 3.8 times more likely to be HSV-2 seropositive; and those who reported a coital debut before age 15 were 2.1 times more likely to be HSV-2 seropositive, although this association did not reach statistical significance (Table).

Educational level, category of HIV acquisition, past STD history, and CD4 count category did not appear to be associated with HSV-2. There was a non-significant inverse association between HSV-2 seropositivity and increasing age.

Associations with the same potential risk factors were also examined separately by gender. Only "lifetime number of sexual partners" among men was associated with HSV-2 infection, with significant linear trend (p = 0.041). Men who reported more than 10 sexual partners in their lifetime were 3.5 times more likely to be HSV-2 seropositive (OR 3.6; 95% CI: 0.7 - 19.9).

Sixty (47.2%) of the 127 subjects who gave no history of genital herpes had HSV-2 detectable antibodies. Sixty-six (84.6%) of the 78 subjects with HSV-2 antibodies did not report a history of genital herpes.

DISCUSSION

The HSV-2 seroprevalence of 52% observed in our study population is higher than that reported in antenatal clinic attenders (29%; mean age: 24.9 years) and blood donors (26%; mean age: 37.4 years) in Brazil (Weinberg et al. 1993, Lupi 1995, 1998, Cowan et al. 2003), supporting the strong association between the two infections. According to Russel et al. (2001), the higher rate of HSV-2 seen in HIV-antibody positive subjects may indicate that individuals who are infected by HSV are more likely to acquire HIV infection, and vice-versa.

Limited data on HSV-2 prevalence among HIV-positive patients are available from Brazil. Lupi (1998) found an 87% seroprevalence of HSV-2 in a group of 100 HIV-seropositive, homosexual and bisexual patients, with a history of multiple sexual partnerships. The higher rates of infection observed in that study could have resulted from different distribution of risk factors in these populations, mainly related to high levels of sexual activity. High rates of HSV-2 infection among HIV seropositive patients have also been reported from other countries: 62.7 - 100% in the US (Stamm et al. 1988, Nahmias et al. 1990, Safrin et al 1991, Hook et al. 1992), 64% in the United Kingdom (Allan & Das 2004), 88% in Haiti (Boulos et al. 1992), and 91% in Central African Republic (Mbopi-Kéou et al. 2000). Seroprevalence among HIV seronegative subjects varies widely across regions, reaching levels comparable to those in HIV seropositive patients where early and high levels of sexual exposure are recorded (Whitley & Gnann 1993).

Other studies have shown that the risk of HSV-2 infection increases with age, low education levels, premature sexual initiation, STD history, and multiple sexual partnerships (Mertz 1993, Santos et al. 1995, Lupi 1998, Suligoi et al. 2001). However, in our study, only age of coital debut and lifetime numbers of sexual partners among men were significantly associated with higher HSV-2 seropositivity. Our results contrast with those findings suggesting that other factors may contribute to increased HSV-2 seropositivity in HIV positive patients. It is unclear to what extent HIV infection shapes the profile of HSV-2 infected subjects. The inverse association with age is counterintuitive since seropositive accumulates as individuals grow older. The probability that it has resulted from chance is not trivial, but one might speculate that HSV-2 infection changes the survival of HIV-positive individuals. If age is a proxy of time of HIV infection, a lower seroprevalence of

TABLE

Unvariate analysis of risk factors for herpes simplex virus type 2 (HSV-2) infection among human immunodeficiency virus infected patients in Niterói, Rio de Janeiro

Characteristic	N	N (%) HSV-2+	O.R. (95% C.I.)	p-value
Gender				0.7825[a]
Male	83	44 (53.0)	1.00	
Female	67	34 (50.7)	0.91 (0.48 - 1.74)	
Age groups (years)				0,1853[a]
20-29	34	21 (61.8)	1.00	0,0716[b]
30-39	58	32 (55.2)	0.76 (0.29 - 1.97)	
≥ 40	58	25 (43.1)	0.47 (0.20 - 1.11)	
Education level				0.4836[a]
Elementary [c]	81	51 (63.0)	1.70(0.51 - 5.66)	0.2350[b]
High school	53	29 (54.7)	1.21 (0.34 - 4.25)	
College	16	8 (50.0)	1.00	
Age (years) of first sexual intercourse				0.048[a]
< 15	50	33 (66.0)	2.15 (0.84 - 5.52)	0.1664[b]
15-17	60	26 (43.3)	0.85 (0.35 - 2.04)	
≥ 18	40	19 (47.5)	1.00	
Lifetime number of sexual partners				0.2402[a]
≤ 3	49	22 (44.9)	1.00	0.1017[b]
4 - 10	36	17 (47.2)	1.10 (0.42 - 2.85)	
> 10	60	36 (60.0)	1.84 (0.80 - 4.25)	
Unknown	5	3 (60.0)		
HIV acquisition				0.5894[a]
Transfusion	8	3 (37.5)	0.52 (0.09 - 2.74)	
IDU	3	2 (66.7)	1.74 (0.12 - 50.57)	
MSM	39	24 (61.5)	1.40 (0.60 - 3.24)	
Heterosexual	88	47 (53.4)	1.00	
Unknown	12	2 (16.7)		
Past STD history				0.7932[a]
Yes	51	28 (54.9)	1.10 (0.52 - 2.30)	
No	95	50 (52.6)	1.00	
Unknown	4	0 (-)		
Past history of genital herpes				0.0093[a]
Yes	22	17 (77.3)	3.80 (1.22 - 12.61)	
No	12	60 (47.2)	1.00	
Unknown	71	1 (100.0)		
CD4 cell count/mm^3				0.7842[a]
> 350	59	28 (47.5)	1.00	0.5090[b]
200-350	32	14 (43.8)	0.86 (0.33 - 2.23)	
50-199	26	14 (53.9)	1.29 (0.46 - 3.61)	
< 50	12	7 (58.3)	1.55 (0.38 - 6.48)	

a: Pearson chi-square; *b*: trend chi-square; *c*: including one case illiterate; IDU: intravenous drug user; MSM: men who have sex with men; STD: sexually transmitted disease.

HSV-2 would be consistent with lower survival.

Studies in HIV-positive individuals have demonstrated that asymptomatic genital HSV-2 shedding increases with lower CD4 counts (Augenbraun et al. 1995, Wright et al. 2003). In addition, HIV-positive subjects are more likely to have symptomatic HSV-2 disease, including more frequent and more severe mucocutaneous outbreaks (Stewart et al. 1995). These data have important implications with regard to the transmission of both virus. We did not study HSV-2 shedding in our HIV-positive patients. Our results showed a weak and statistically non significant association of HSV-2 and CD4 cell count. Evidence that HSV-2-infection increases the risk of transmitting HIV (Celum 2004) indicates that further studies are necessary to clarify the interaction of these two infections.

Smith et al. (2001) found that middle-age women in Brazil that suspected their husbands had extramarital partners had higher HSV-2 seropositivity rates. According to these authors, this finding suggests that the risk of HSV-2 acquisition is dependent not only on whether a woman's husband has other sexual partners, but also on the likelihood that he will acquire HSV-2 infection from these sexual partners. Although this factor has not been investigated in our study, it is possible that it could have contributed

to the high HSV-2 seroprevalence found among women. This is consistent with the high proportion (55.9%) of HSV-2 positive women who reported fewer than four lifetime sexual partners.

Our results showed that although HSV-2 antibodies were quite frequent in the study population, clinical expression of the infection was rather uncommon. History of genital herpes was obtained from 21.8% of the HSV-2 positive subjects, which indicates that only a low proportion of the patients were aware of the HSV-2 infection. This finding is in accordance with other reports (Weinberg et al. 1993, Russel et al. 2001, van Benthem et al. 2001), confirming 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 herpes (Langenberg et al. 1989, Weinberg et al. 1993) and counseling them to seek health care for genital signs and symptoms. The potential role of HSV-2 infection in facilitating HIV transmission highlights the need for including anti-HSV-2 testing and therapy in the management of HIV positive patients, especially for reducing the risk of transmission of HIV through herpetic lesions (Suligoi et al. 2002).

We recognize some limitations to our study. The results were based on cross-sectional data from HIV-positive patients representing the more severe forms of the clinical spectrum of the disease. Nevertheless, HSV infection is thought to precede, and might increase the odds of HIV infection. As a result of the interaction of those infections, the frequency and severity of genital herpes and virus shedding may be modified. In addition to that limitation, current data are based on HIV-positive patients selected from a public hospital and may not have been representative of the HIV-positive population of the municipalities of Niteroi and São Gonçalo where most patients come from. These selection factors limit the generalizability of the estimates of HSV-2 infection rates. This was an exploratory study based on data available from a limited number of patients. The study lacked statistical power to demonstrate the association of HSV-2 infection with factors such as age of first sexual intercourse, which is suggested by the magnitude of the odds ratio (2.15). Weak associations and the sample size limited the control of confounding through multivariate analysis. Follow-up data comparing HSV-2 seropositive and seronegative subjects were not available to allow the analysis of the effect of co-infection HIV-HSV-2. Failure of patients to provide accurate information to questions related to sexual behaviour and drug use may have biased our estimates to the null as non differential misclassification is more likely. However, our study has recorded very high rate of HSV-2 infection, which may affect the natural history of HIV/AIDS and enhance the transmissibility of both virus (van Benthem et al. 2001). Further studies should clarify the implications of the co-infection for HIV-patients follow-up and prognosis.

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REFERENCES

Allan PS, Das S 2004. Prevalence of HSV-1/HSV-2 antibodies in HIV seropositive patients in Coventry, United Kingdom. *Sex Transm Infect 80*: 77.

Ashley RL, Corey L, Rawls WE 1990. Herpes simplex viruses. In NJ Schimidt, RW Emmens (eds), *Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections*, 6th ed., American Public Health Association, Washington D.C, cap.11, p. 265-317.

Ashley RL, Wu L, Pickering JW, Tu MC, Schnorenberg L 1998. Premarket evaluation of a commercial glycoprotein G-based enzyme immunoassay for herpes simplex virus type-specific antibodies. *J Clin Microbiol 36*: 294-295.

Augenbraun M, Feldman J, Chirgwin K, Zenilman J, Clarke L, DeHovitz J, Landesman S, Minkoff H 1995. Increased genital shedding of herpes simplex type 2 in HIV-1 seropositive women. *Ann Intern Med 123*: 845-847.

Boulos R, Ruff AJ, Nahmias A, Holt E, Harrison L, Magder L, Wiktor SZ, Quinn TC, Margolis H, Halsey NA 1992. Herpes simplex virus type 2 infection, syphilis, and hepatitis B infection in Haitian women with human immunodeficiency virus type 1 and human T lymphotropic virus type 1 infection. *J Infect Dis 166*: 418-420.

Celum CL 2004. The interaction between herpes simplex virus and human immunodeficiency virus. *Herpes 11* (Suppl. 1): 36A-45A.

Chen CY, Ballard RC, Beck-Sague CM, Dangor Y, Radebe F, Schmid S, Weiss JB, Tshabalala V, Fehler G, Htun Y, Morse SA 2000. Human immunodeficiency virus infection and genital ulcer disease in South Africa: the herpetic connection. *Sex Transm Dis 27*: 21-29.

Corey L, Handsfield HH 2000. Genital herpes and public health - addressing a global problem. *JAMA 283*: 791-794.

Cowan FM, French RS, Mayaud P, Gopal R, Robinson NJ, Artimos de Oliveira S, Faillace T, Uusküla A, Nigard-Kibur M, Ramalingam S, Sridharan G, El Aouad R, Alami K, Rbai M, Sunil-Chandra NP, Brown DW 2003. Seroepidemiological study of herpes simplex virus types 1 and 2 in Brazil, Estonia, India, Morocco, and Sri Lamka. *Sex Trans Infect 79*: 286-290.

Hook EW, Cannon RD, Nahmias AJ, Lee FF, Campbell Jr. CH, Glasser D, Quinn TC 1992. Herpes simplex virus as a factor for human immunodeficiency virus infection in heterosexuals. *J Infect Dis 165*: 251-255.

Langenberg A, Benedetti J, Jenkins J, Ashley R, Winter C, Corey L 1989. Development of clinically recognizable genital lesions among women previously identified as having "asymptomatic" herpes simplex virus type 2 infection. *Ann Intern Med 110*: 882-887.

Lupi O 1995. *Fatores de Risco na Aquisição do Herpes Simples do Tipo 2 (HSV-2) em Populações de Baixo e Alto Risco*

para Doenças Sexualmente Transmissíveis, Thesis, Faculdade de Medicina, Universidade Federal do Rio de Janeiro, 101 pp.

Lupi O 1998. *Herpes Simples e Infecção pelo Vírus da Imunodeficiência Humana: Análise de 650 Pacientes*, Thesis, Faculdade de Medicina, Universidade Federal do Rio de Janeiro, 153 pp.

Mbopi-Kéou FX, Grésenguet G, Mayaud P, Weiss HA, Gopal R, Matta M, Paul JL, Brown DWG, Hayes RJ, Mabey DCW, Bélec L 2000. Interactions between herpes simplex virus type 2 and immunodeficiency virus type 1infection in African women: opportunities for intervention. *J Infect Dis 182*: 1090-1096.

McGrath BJ, Newman CL 1994. Genital herpes simplex infections in patients with the acquired immunodeficiency syndrome. *Pharmacotherapy 14*: 529-542.

Mertz GJ 1993. Epidemiology of genital herpes infections. *Infect Dis Clin North Am 7*: 825-839.

Nahmias AJ, Lee FK, Beckman-Nahmias S 1990. Seroepidemiological and sociological patterns of herpes simplex virus infection in the world. *Scand J Infect Dis 69*: 19-36.

Russel DB, Tabrizi SN, Russel JM, Garland SM 2001. Seroprevalence of herpes simplex virus types 1 and 2 in HIV-infected and uninfected homosexual men in a primary care setting. *J Clin Virol 22*: 305-313.

Safrin S, Ashley R, Houlihan C, Cusick PS, Mills J 1991. Clinical and serological features of herpes simplex virus infection in patients with AIDS. *AIDS 5*: 1107-1110.

Santos OLR, Silva AG, Pereira Jr AC 1995. Herpes simples genital. Uma doença sexualmente transmissível. *F Méd (BR) 110*: 77-98.

Smith JS, Herrero R, Muñoz N, Eluf-Neto J, Ngelangel C, Xavier Bosch F, Ashley RL 2001. Prevalence and risk factors for herpes simplex vírus type 2 infection among middle-age women in Brazil and the Philippines. *Sex Transm Dis 28*: 187-194.

Stamm W, Handsfield HH, Rompalo AM, Ashley RL, Roberts PL, Corey L 1988. The association between genital ulcer disease and acquisition of HIV infection in homosexual men. *JAMA 260*: 1429-1450.

Stewart JA, Reef SE, Pellett PE, Corey L, Whitley RJ 1995. Herpesvirus infections in persons infected with human immunodeficiency virus. *Clin Infect Dis 21* (Suppl. 1): S114-120.

Suligoi B, Tchamgmena O, Sarmati L, Bugarini R, Toma L, Bakary DK, Glikoutou M, Rezza G 2001. Prevalence and risk factors for herpes simplex vírus type 2 infection among adolescents and adults in Northern Cameroon. *Sex Transm Dis 28*: 690-693.

Suligoi B, Dorrucci M, Volpi, A, Andreoni M, Zerboni R, Rezza G and The Italian Seroconversion Study Group 2002. Prevalence and determinants of herpes simples virus type 2 infection in a cohort of HIV-positive individuals in Italy. *Sex Trans Dis 15*: 665-667.

Turner KR, MaFarland W, Kellogg TA, Wong E, Page-Shafer K, Louie B, Dilley J, Kent CK, Klausner J 2003. Incidence and prevalence of herpes simplex virus type 2 infection in persons seeking repeat HIV counseling and testing. *Sex Transm Dis 30*: 331-334.

van Benthem BHB, Spaargaren J, van den Hoek JAR, Merks J, Coutinho RA, Prins M, European Study on the Natural History of HIV Infection in Women 2001. Prevalence and risk factors of HSV-1 and HSV-2 antibodies in European HIV infected women. *Sex Transm Infect 77*: 120-124.

Wald A, Link K 2002. Risk of human immunodeficiency virus infection in herpes simplex virus type 2 seropositive persons: a meta-analysis. *J Infect Dis 198*: 45-52.

Wasserheit J 1992. Epidemiological synergy: interrelationships between human immunodeficiency virus infection and other sexually transmitted diseases. *Sex Transm Dis 19*: 61-77.

Weinberg A, Canto CLM, Pannutti CS, Kwang WN, Garcia SAL, Zugaib M 1993. Herpes simplex vírus type 2 infection in pregnancy: asymptomatic viral excretion at delivery and seroepidemiologic survey of two socioeconomically distinct populations in São Paulo, Brazil. *Rev Inst Med Trop São Paulo 35*: 285-290.

Whitley RJ, Gnann JW 1993. The epidemiology and clinical manifestations of herpes simplex virus infections. In B Roizman, RJ Whitley, C Lopez (eds), *The Human Herpesviruses*, Raven Press, New York, p. 69-105.

WHO and UNAIDS 2001. Herpes simplex virus type 2: programmatic and research priorities in developing countries. Report of a WHO/UNAIDS/LSHTM workshop. London, 14-16 February. WHO_HIVAIDS/2001.05.2001.

Wright PW, Hoesley CJ, Squires KE, Croom-Rivers A, Weiss HL, Gnann Jr JW 2003. A prospective study of genital herpes simplex virus type 2 infection in human immunodeficiency virus type 1 (HSV-1)-seropositive women: correlations with CD4 cell count and plasma HIV-1 RNA level. *Clin Infect Dis 36*: 207-211.