# Human Herpesvirus-8 (HHV-8) Antibodies in Women From São Paulo, Brazil. Association With Behavioral Factors and Kaposi's Sarcoma

**Adele Caterino-de-Araujo[1], Elizabeth de los Santos-Fortuna[1], Paulo Henrique Lage Carbone[2], Sandra Elisa Lopes Cibella[1] and Abdiel Aparecido Moreira[2]**

*Immunology Department, Instituto Adolfo Lutz, Secretary of Health of São Paulo[1], University of São Paulo[2], SP, Brazil*

**Background: With the spread of AIDS, many HIV-infected women have been diagnosed with Kaposi's sarcoma (KS), especially in Africa. Since the discovery of a novel herpesvirus as the causative agent of KS (human herpesvirus 8 – HHV-8) several seroepidemiological studies have been conducted to identify groups at risk for KS. The risk for women in Brazil has not been studied. Materials and Methods: We searched for HHV-8 antibodies in sera obtained from a bank made up of samples from 3 groups of individuals: Group I: 163 HIV-1-infected women attended at an ambulatory clinic in 1994; Group II: 108 children born to HIV-1-infected mothers from 1990 to 1992, their antibodies reflected maternal infection, and Group III: 630 HIV-1-seronegative, healthy women. In-house immunofluorescence and Western-Blot assays based on the BCBL-1 cell line were used to detect anti-latent and anti-lytic HHV-8 antibodies. Results: Group I had an overall frequency of antibodies of 8.6%, with a 1.2% frequency of anti-latent antibodies and an 8.0% frequency of anti-lytic antibodies. Similar results were detected in Group II, i.e., no cases with anti-latent antibodies and a 7.4% frequency of anti-lytic antibodies. In contrast, prevalences of 1.1% anti-latent antibodies and 0.3% anti-lytic antibodies were observed in Group III. Conclusions: The epidemiologic pattern of HHV-8 in women from São Paulo varies according to behavioral factors, with emphasis on the sexual and blood routes of virus transmission/acquisition. Although HHV-8 anti-lytic antibodies were found in HIV-1-infected women, no case of KS was detected. Protective factors against KS are probably related to gender and/or to antiretroviral therapies introduced in Brazil since 1994.**

**Key Words: Human herpesvirus – 8 (HHV-8) anti-lytic and anti-latent antibodies, HHV-8 infection in women, HHV-8 in HIV-1 infected women, HHV-8 seroprevalence, HHV-8 infection, Kaposi's sarcoma (KS).**

**Abbreviations: Human herpesvirus – 8 (HHV-8); Kaposi's sarcoma (KS); Kaposi's sarcoma-associated with HIV-1 infection (KS-AIDS); Immunofluorescence assay (IFA); Latent nuclear antigen (LANA); Western-Blot (WB); Body cavity-based lymphoma cell line (BCBL-1); highly active antiretroviral therapy (HAART); injectable drug users (IDUs); human T-cell leukemia/ lymphoma type I (HTLV-I); intravenous drug users (IVDU); primary effusion lymphoma (PEL); antibodies (Ab).**

Received on 08 May 2003; revised 01 September 2003.
Address for correspondence: Dr..Adele Caterino-de-Araujo. Seção de Imunologia, Instituto Adolfo Lutz. Av. Dr. Arnaldo, 355, 11º andar, 01246-902, São Paulo, SP, Brazil.; telephone/ fax: 0055-11-30682898. E-mail: caterino@ial.sp.gov.br, caterino@usp.br.

**The Brazilian Journal of Infectious Diseases** 2003;7(6):395-401


The distribution of human herpesvirus-8 (HHV-8) infection around the world mirrors the epidemiology of Kaposi's sarcoma (KS), with areas with a high incidence of KS having the highest HHV-8 prevalences [1-2]. Nevertheless, exceptions have been found in some areas of West Africa, South America and Australasia [1,3-5]. Although these regions are considered endemic for HHV-8 infection, few or no

cases of KS have been detected in them. Several factors affect these findings, including ethnic background of the individuals and environmental conditions.

Interestingly, in endemic populations, no gender differences in HHV-8 seroprevalence have been detected, although historically KS has occurred less frequently among females than males [1-2]. Female hormones, such as human chorionic gonadotropin and luteinizing hormone, may play a role in KS avoidance [6]. Other factors have been pointed out as regulators of KS outcome, such as the interruption of post-transplant immunosuppressive therapy in KS, and the use of highly active antiretroviral therapy (HAART) by HIV-infected individuals [7-8].

A series of studies have found that sexual contact is the major route of HHV-8 transmission, mostly in patients at risk for HIV infection and in patients with KS-AIDS. On the other hand, saliva and/or other body fluids have been considered to be vehicles for the spread of virus in patients presenting classic and endemic KS, as well as in healthy populations in areas endemic for HHV-8 infection, especially during childhood [1-5]. Studies conducted on women worldwide have produced mixed findings, showing higher incidences among female injectable drug users (IDUs) in the US, females who have HIV-infected partners, female sex workers, and females living in KS-endemic regions, such as Africa and the Caribbean [2, 9-10]. To our knowledge, no study has been conducted in Brazil on a large number of women, except for the Amerindian population [4].

## Materials and Methods

Since 1990, we have been doing population-based seroepidemiological surveys of groups at risk for HIV-infection and/or infected with HIV/AIDS from São Paulo, Brazil. Our initial aims were to characterize the epidemiology of HIV in children born to HIV-infected mothers and to study human T-cell leukemia/lymphoma I (HTLV-I) and HTLV-II in children and in adults infected or not with HIV/AIDS [11-13]. We decided to use serum samples from cohorts still available for analysis in order to add some information concerning HHV-8 infection among high-risk groups of women. The selected groups were: Group I - Serum samples from 163 HIV-1-infected women seen at Instituto de Infectologia Emílio Ribas in 1994, receiving medical and therapeutic care for opportunistic infections as well as antiretroviral therapy, first with transcriptase inhibitors, and beginning in 1996, also with protease inhibitors; they were tested for HHV-8 antibodies. Based on epidemiological and clinical data, 123 of the patients had sexual risk factors (122 were heterosexual, and one was homosexual), 36 had blood-borne risk factors (22 were intravenous drug users (IVDU), and 4 had multiple blood transfusions), and 14 women had unknown risk factors. The median age of the women was 31 years, ranging from 22 to 44 years. After a 5-year follow-up, the group was examined for HHV-8-associated diseases. No case of KS or primary effusion lymphoma (PEL) was detected in their medical records. Group II: Plasma samples from 108 children under 2 years of age, born to HIV-1-infected mothers and in whom HIV-1 vertical transmission was studied over a 3-year period (1990 to 1993), were analyzed for maternal HHV-8 antibodies that crossed the placenta [14]. After a 2-year follow-up the HIV-1 status of these children showed 55 HIV-1-infected subjects, 29 HIV-1-non-infected subjects, and 24 cases of undefined HIV-1 status (cases of abandonment and/or those who had made only one medical visit and from whom only one blood sample was collected). The epidemiological data of the mothers revealed a median age of 20 years (range, 16-24 years), and IVDU was the main risk factor for acquiring HIV (80% of the cases), followed by prostitution and/or sexual contact with HIV-infected partners.

In addition, we used sera from a bank of the Immunology Department of Instituto Adolfo Lutz in order to compare the results obtained for groups at high-risk and at low-risk to acquire HIV. Group III: Serum samples collected in 1997 from 630 normal healthy women working at an institution that takes care of mentally handicapped persons in São Paulo, and who were submitted to a program of vaccination against hepatitis B, were also tested for HHV-8 antibodies. The median age of the participants was 40 years, ranging

from 20 to 74 years. Most of them had been working at this institution for a long time, and no one had AIDS or KS.

Antibodies to latent nuclear and lytic HHV-8 antigens were detected using two different in-house immunofluorescence (IFA) and Western-Blot assays based on the BCBL-1 cell line. The cells were grown in RPMI 1640 containing 10% heat-inactivated fetal calf serum (FCS) and antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin).

For IFA of anti-latent nuclear antigens (IFA-LANA), the cells were washed twice with phosphate buffered saline (PBS), and resuspended in PBS to obtain a concentration of $4x10^6$ cells/ml. A 10 µl suspension was smeared on slides, air dried at room temperature, and fixed with acetone at –20°C for 20 minutes.

For IFA to anti-lytic antibodies (IFA-Lytic), smears were similarly prepared by sedimenting BCBL-1 cells, after treatment with 20ng/ml tetradecanoyl phorbol ester acetate.

Fixed smears were preblocked by incubation with PBS supplemented with non-fat milk for 10 minutes at 37°C in a humidified chamber. The smears were incubated in two 30-minute steps at 37°C with the test serum diluted 1:50 in PBS-milk and with a goat anti-human antibody fluorescein isothiocyanate-conjugate (Sigma, 1:500 in PBS-Evans blue-milk). Titrations were made with two-fold serial dilutions, and the samples were considered positive when they were reactive at a dilution of at least 1:50.

For the confirmation of IFA-LANA positive results, all the samples were run on p226-234 Western-Blots. Briefly, the protein extract obtained by washing BCBL-1 cell line in PBS, and subsequent lysis at –20°C in distilled water was run in 10% SDS-PAGE and blotted onto a nitrocellulose membrane (Bio-Rad). After blocking with 5% PBS-milk, membrane strips were incubated overnight with samples diluted 1:100 in PBS-milk. After 3 x washing with PBS, blots were incubated with anti-human IgG peroxidase-conjugate (Sigma, 1:2,000 in PBS-milk 1%), and after subsequent 3 x washing, developed with 4-chloro 1-naftol (Sigma) and hydrogen peroxide (Sigma). Samples reacting with a typical antigen doublet with a molecular weight of 226-234 kDa were considered to be WB-LANA positive. We confirmed the sensitivity and specificity of these in-house assays in our laboratory, with the detection of HHV-8 anti-latent antibodies and anti-lytic antibodies, respectively, in 75.1% and 90.9% of the KS-AIDS patients (44 cases), but in only 0.95% and 0.3% among healthy persons (736 individuals) from São Paulo, Brazil (Caterino-de-Araujo et al., unpublished data).

Informed consent was obtained from the patients, and from the parents of the children. The study was conducted in accordance with the Human Experimentation Guidelines of Instituto de Infectologia Emílio Ribas, and Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, SP, Brazil. Informed consent to use the serum samples from the group of individuals working at the institution that takes care of handicapped persons in São Paulo was obtained from their legal representatives, in accordance with the Experimentation Guidelines and with the Ethics Committee of this Institution.

## Results

Group I presented an overall HHV-8 antibody frequency of 8.6%, a 1.2% frequency of anti-latent antibodies and an 8.0% frequency of anti-lytic antibodies. Depending on the risk factor for acquiring HIV-infection, these frequencies ranged from 10.7% to 0%. The highest percentage of HHV-8 seropositivity was observed among women with a heterosexual risk factor, followed by IVDU (4.5%). No case of HHV-8 seropositivity was detected among blood-transfused women or among women with unknown risk factors (Table 1). The characteristics of the study group, together with the results obtained by IFA-LANA and IFA-Lytic, are presented in Table 2. Titers of 1:100 to 1:3,200 of anti-latent antibodies and anti-lytic antibodies were detected.

In Group II, an overall frequency of 7.4% HHV-8 antibodies was detected among children born to HIV-infected mothers, ranging from 10.9% to 0%. A 10.9% frequency of HHV-8 antibody was detected among

HIV-infected children, and an 8.3% frequency was observed in children with undefined HIV status. No case of HHV-8 antibody was detected in HIV-non-infected children (Table 1). Table 2 presents the individual positive results obtained by HHV-8 serology, showing only HHV-8 anti-lytic antibodies, ranging in titers from 1:100 to 1:800. Both boys and girls had received maternal antibodies through the placenta.

In Group III, antibodies to HHV-8 were detected in 1.3% of the cases: HHV-8 anti-latent antibodies were detected in 1.1% of the cases and anti-lytic antibodies in 0.3% (Table 1). Titers ranging from 1:50 to 1:800 to HHV-8 anti-latent antibodies were observed (Table 2).

## Discussion

The results obtained for HIV-infected women from São Paulo, Brazil, belonging to Group I, point to the sexual route as the most important one for acquiring HHV-8, although blood-borne infection could not be excluded. This conclusion has also been made in most of the studies conducted around the world on the main risk factors for women to acquire HHV-8 [1,2,7,8, 10]. On the other hand, if we consider the antibodies detected in children to be maternal antibodies, we can identify the intravenous (blood) route as another route of virus transmission and/or acquisition.

Even if these results seem to be contrasting, we must consider the time when these samples were collected. At the beginning of the AIDS epidemic in São Paulo, most female cases involved young IVDU, whereas AIDS was later detected in older heterosexual women who had sexual contact with HIV/AIDS bisexual men. So, the results obtained agree follow the epidemiology of AIDS in São Paulo, and emphasize another route of HHV-8 transmission, the intravenous one, the same route pointed out by another study conducted in the United States on women with, or at risk for, HIV infection [9].

In spite of the frequent detection of HHV-8 anti-lytic antibodies in both groups (which reflects virus activation/replication), plus the immunodepression characteristic of HIV/AIDS patients, no case of KS was detected in Group I or Group II, even during the follow-up period. These data concerning women could be explained by the use of antiretroviral therapy in São Paulo since 1994, and the female hormones secreted during menstruation, and among children, by the presence of maternal antibodies and no true HHV-8 infection. No case of KS was detected among mothers at delivery, suggesting a protective effect of the pregnancy hormones. Unfortunately, we did not have access to the HIV staging of the mothers during the follow-up of the children, but we did find that mothers who are capable of transmitting HIV to their offspring are more likely to maintain HHV-8 infection (Table 1). This could be a direct or an indirect effect of HIV promoting HHV-8 viral growth. None of the children born to HIV-infected mothers were breast-fed, with consequent avoidance of HTLV-I, HTLV-II, and probably HHV-8, vertical transmission [12,14].

Even though the number of KS-AIDS cases diminished after the introduction of antiretroviral therapy in São Paulo [15], no large changes in HHV-8 antibody frequencies were detected among the women treated (Group I) or not (Group II) with HAART.

Finally, the results obtained for Group III confirm that HHV-8 also circulates among women at low-risk to acquire HIV/AIDS in São Paulo, Brazil, and shows that in this population HHV-8 remains in latency until various factors contribute to its reactivation.

## Acknowledgements

This research was supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) grant # 98/13313-5. PHL Carbone is the recipient of a fellowship from the University of São Paulo, supported by the Conselho Nacional de Pesquisa e Desenvolvimento (CNPq), SEL Cibella is the recipient of a fellowship from Instituto Adolfo Lutz supported by Fundação de Desenvolvimento Administrativo (FUNDAP), and AA Moreira is the recipient of a fellowship from the University of São Paulo, supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

**Table 1**. Frequency rates of HHV-8 anti-latent and anti-lytic antibodies among HIV-1-infected women (Group I), among children born to HIV-1 infected mothers (Group II), and among normal healthy women from São Paulo, Brazil

| Group of subjects | No. of cases tested | IFA and WB LANA n (%) | IFA-Lytic n (%) | HHV-8 antibodies* n (%) |
|---|---|---|---|---|
| **Group I** | **163** | **2 (1.2)** | **13 (8.0)** | **14 (8.6)** |
| Hetero | 122 | 2 (1.6) | 12 (9.8) | 13 (10.7) |
| Homo | 1 | - | - | |
| IVDU | 22 | - | 1 (4.5) | 1 (4.5) |
| Polytransfused | 4 | - | - | - |
| Unknown | 14 | - | - | - |
| **Group II** | **108** | **-** | **8 (7.4)** | **8 (7.4)** |
| $HIV^+$ | 55 | - | 6 (10.3) | 6 (10.3) |
| $HIV^-$ | 29 | - | - | - |
| HIV undefined | 24 | - | 2 (8.3) | 2 (8.3) |
| **Group III** | **630** | **7 (1.3)** | **2 (0.3)** | **8 (1.5)** |

Risk factor to acquire HIV-1: hetero (heterosexual contact), homo (homosexual contact), IVDU (intravenous drug user). * Overall frequency of HHV-8 antibodies according to the group analyzed.

This work was partially presented at the XIV International AIDS Conference, July 2002, Barcelona, Spain., and at the FeSBE 2002 Meeting (Federação de Sociedades de Biologia Experimental), August 2002, Salvador, BA, Brazil.

## References

1. Schulz T.F. Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8). J Gen Virol **1998**;79:1573-91.
2. Spira T.J., Jaffe H.W. Human herpesvirus 8 and Kaposi's sarcoma. In: Scheld WM, Craig WA, Highes JM, eds. Emerging Infection 2. Washington, D.C., ASM Press, **1998**:Chapter 4:81-105.
3. Plancoulaine S., Abel L., van Beveren M., et al. Human herpesvirus 8 transmission from mother to child and between siblings in an endemic population. Lancet **2000**;356:1062-5.
4. Biggar R.J., Whitby D., Marshall V., et al. Human herpesvirus 8 in Brazilian amerindians: a hyperendemic population with a new subtype. J Infect Dis **2000**;181:1562-8.
5. Rezza G., Danaya R.T., Wagner T.M., et al. Human herpesvirus-8 and other viral infections, Papua New Guinea. Em Infect Dis **2001**;7: 893-5.
6. Lunardi-Iskandar Y., Bryant J.L., Zeman R.A., et al. Tumorigenesis and metastasis of neoplastic Kaposi's sarcoma cell line in immunodeficient mice blocked by a human pregnancy hormone. Nature **1995**;375:64-8.
7. Antman K., Chang Y.. Kaposi's sarcoma. N Eng J Med **2000**;342:1027-38.
8. Aboulafia D.M. The epidemiologic, pathologic, and clinical features of AIDS-associated pulmonary Kaposi's sarcoma. Chest **2000**;117:1128-45.
9. Cannon M.J., Dollard S.C., Smith D.K., et al. Blood-borne and sexual transmission of human herpesvirus 8 in women with or at risk for human immunodeficiency virus infection. N Engl J Med **2001**;344:637-43.
10. Greenblatt R.M., Jacobson L.P., Levine A.M., et al. Human herpesvirus 8 infection and Kaposi's sarcoma among human immunodeficiency virus-infected and –uninfected women. J Infect Dis **2001**;183:1130-4.
11. Caterino-de-Araujo A., Casseb J., Hendry M.R. Use of V3 loop peptide-specific antibody evaluation for subtyping HIV-1: Results of a vertical transmission study from São Paulo, Brazil. J Trop Pediatr **1999**;45:265-70.
12. Caterino-de-Araujo A., Santos-Fortuna E. No evidence of vertical transmission of HTLV-I and HTLV-II in children at high risk for HIV-1 infection from São Paulo, Brazil. J Trop Pediatr **1999**;45:42-7.

**Table 2.** Characteristics and individual results obtained using HHV-8 serology to detect anti-latent and anti-lytic antibodies in HIV-1-infected women (Group I), in children born to HIV-1-infected mothers (Group II), and in normal healthy women (Group III) from São Paulo, Brazil

| Subject code | Epidemiological data | | IFA-LANA* | IFA-Lytic* |
|---|---|---|---|---|
| **Group I** | **Age/risk** | **CDC class** | **Ab titer** | **Ab titer** |
| 1- HHS | 33/hetero | AIDS | - | 1:200 |
| 41- MFGO | 30/hetero | GII | 1:800 | 1:800 |
| 89- RMS | 22/hetero | GII | - | 1:400 |
| 149- CR | 35/hetero | GII | - | 1:200 |
| 283- JAP | 29/hetero | AIDS | - | 1:400 |
| 285- EJR | 44/hetero | AIDS | - | 1:1,600 |
| 318- DPS | 23/hetero | GII | - | 1:100 |
| 353- ECS | 24/IVDU | AIDS | - | 1:100 |
| 389- RM | 33/hetero | GII | - | 1:200 |
| 393- MJF | 34/hetero | AIDS | - | 1:100 |
| 435- EPS | 25/hetero | GII | - | 1:3,200 |
| 448- MRWD | 36/hetero | AIDS | 1:100 | - |
| 530- MCS | 29/hetero | GII | - | 1:100 |
| 547- MCF | 34/hetero | AIDS | - | 1:100 |
| **Group II** | **Age/sex** | **HIV status** | **Ab titer** | **Ab titer** |
| 14 | 11/F | $HIV^+$ | - | 1:200 |
| 24 | 16/M | $HIV^+$ | - | 1:200 |
| 53 | 7/F | $HIV^+$ | - | 1:800 |
| 108 | 1.5/M | HIV undefined | - | 1:200 |
| 128 | 24/F | $HIV^+$ | - | 1:100 |
| 138 | 12/M | $HIV^+$ | - | 1:400 |
| 139 | 11/M | $HIV^+$ | - | 1:200 |
| 149 | 5/M | HIV undefined | - | 1:100 |
| **Group III** | **Age/color** | **Time of work** | **Ab titer** | **Ab titer** |
| 256/10023F | 30/M | 05 | 1:100 | 1:800 |
| 851/12127F | 21/W | 01 | 1:50 | - |
| 986/8125F | 42/W | 07 | 1:100 | - |
| 1068/7124F | 40/M | 12 | 1:200 | - |
| 1109/10850F | 35/W | 03 | 1:800 | - |
| 1169/11094F | 37/W | 03 | 1:50 | - |
| 1308/397F | 64/W | 31 | 1:50 | - |

Group I: Age in years; Risk to acquire HIV-1 infection: heterosexual contact and intravenous drug user. Group II: Age in months; sex (male and female); HIV status (infected, uninfected, undefined). Group III. Age in years; color (mulatto, white, and black); years of institutionalized work. * Results are reported as titers of HHV-8 anti-latent and anti-lytic antibodies (Ab titer) obtained by using IFA as explained in Material and Methods. Negative result (-).

13. Caterino-de-Araujo A., Santos-Fortuna E., Zandoná-Meleiro M.C., et al. Sensitivity of two ELISA tests in relation to western blot in detecting HTLV-I and HTLV-II infections among HIV-1 infected patients from São Paulo, Brazil**.** Diagn Microbiol Infect Dis **1998**;30:173-82.
14. Caterino-de-Araujo A., Cibella S.E.L. Searching for antibodies to HHV-8 in children born to HIV-1-infected mothers from São Paulo, Brazil. Relationship to maternal infection. J Trop Pediatr **2003**;49:247-50.
15. Caterino-de-Araujo A., Carbone P.H.L., Martinelli F.L.B., et al. Absence of an association between the presence of human herpesvirus 8 antibodies and the development of Kaposi's sarcoma in HIV-1 infected patients receiving antiretroviral therapy. AIDS **2000**;14:1455-6.