Prevalence of antibodies to human herpesvirus-8 in populations with and without risk for infection in São Paulo State


Abstract

Human herpesvirus 8 (HHV-8) is a newly described herpesvirus that is etiologically associated with all forms of Kaposi’s sarcoma (KS). Seroprevalence studies have shown high prevalence rates of HHV-8 antibodies among men who have sex with men (MSM) and AIDS patients, African children, Brazilian Amerindians, and elderly individuals in certain regions of Europe. The aim of the present study was to determine the prevalence of HHV-8 antibodies in healthy children and young adults from different cities in São Paulo State, and in a population at high risk for HHV-8 infection: HIV-negative MSM, and AIDS patients with and without KS. Antibodies to HHV-8 latency-associated nuclear antigen and lytic-phase antigens were detected by immunofluorescence assays. In 643 healthy children and young adults from the general population attending a vaccination program for yellow fever in ten different cities in São Paulo State, the prevalence of HHV-8 antibodies detected by the presence of latent or lytic antigens ranged from 1.0 to 4.1% in the different age groups (mean = 2.5%). In the MSM group, the prevalence was 31/95 (32.6%). In the group of patients with AIDS, the prevalence was 39.2% (51/130) for non-KS patients and 98.7% (77/78) for AIDS patients with the diagnosis of KS confirmed by histopathological examination. We conclude that HHV-8 has a restricted circulation among healthy children and young adults in the general population of São Paulo State and a high prevalence among MSM and AIDS patients.

Key words
- HHV-8
- Seroprevalence
- AIDS
- Kaposi’s sarcoma
- Immunofluorescence assay
- Latency-associated nuclear antigen
- Lytic-phase antigens
Human herpesvirus 8 (HHV-8), also known as Kaposi’s sarcoma-associated herpesvirus, is a newly described gamma herpesvirus that is etiologically associated with all types of Kaposi’s sarcoma (KS).

Despite important advances in our knowledge about the genetic and molecular aspects of the virus, the various modes of transmission of HHV-8 remain somewhat controversial. Seroepidemiological studies have shown high prevalence rates of HHV-8 antibodies among male homosexuals (1-3), African children (4,5), Brazilian Amerindians (6) and elderly individuals in certain regions of Europe (3,7). However, there is no single route of transmission explaining how the virus can infect such different population groups. In the United States, northern Europe, and Japan studies have indicated that the seroprevalence of this virus is low in the general population (8-10). However, in Mediterranean countries there are areas where relatively high prevalence rates of classic KS and HHV-8 infection have been observed (11). In such areas, HHV-8 infection can occur during childhood and adolescence (12), suggesting that transmission of the virus may occur through some form of nonsexual contact. This is particularly evident in African populations, where high prevalence rates have been observed in children. A prevalence rate of 39.8% was found in 5-10-year-old children in northern Cameroon (5). In Uganda approximately 50% of children before the age of puberty were found to be infected by HHV-8 (4).

Men who have sex with men (MSM), regardless of HIV serostatus, have a higher rate of HHV-8 infection than the general male population. In MSM who are HIV negative and asymptomatic, infection rates have ranged from 16.8 to 22.1% (1,3,13). An epidemiological relationship between male homosexual activities and HHV-8 infection has been demonstrated in studies undertaken in different countries (1-3,8,9,13).

In Brazil, 600,000 people are currently living with AIDS and KS is the most prevalent neoplasm affecting these patients. There are few studies regarding the prevalence of HHV-8 infection. Among blood donors, seroprevalence rates of 4.6 and 7.4% were found in Vitória and São Paulo, respectively (14,15). Among HIV patients without KS, the seroprevalence rates observed in these two studies were 16 and 18.5%, respectively.

The aim of the present study was to evaluate the prevalence of HHV-8 antibodies in individuals without risk for HHV-8 infection (healthy children and young adults from the general population from different cities of São Paulo State) and in individuals at risk for sexually transmitted diseases (healthy HIV-negative MSM and AIDS patients with and without KS).

For assessment of the prevalence of HHV-8 antibodies in the population without risk for HHV-8 infection, sera from 643 healthy children and young adults attending a vaccination program for yellow fever in ten different cities of São Paulo State (Campinas, Jardinópolis, Marília, Ribeirão Preto, Jaboatícabal, Piracicaba, São José do Rio Preto, Bauru, Dracena, and Presidente Epitácio) were examined for HHV-8-specific antibodies. Sera from MSM were obtained from volunteers of the Bela Vista Project, an HIV seroincidence cohort study undertaken in the city of São Paulo to evaluate the rate of HIV infection in this population. Sera from HIV patients with and without KS were obtained from AIDS patients attending the AIDS Clinic of the Department of Infectious Diseases of the University of São Paulo School of Medicine (Casa da AIDS) and from the Instituto de Infectologia Emílio Ribas, a tertiary-care state hospital for infectious diseases.

Antibodies to HHV-8 latency-associated nuclear antigen (LANA) and lytic-phase antigens were detected by previously described immunofluorescence assays (IFA) (16). IFA were performed employing the BCBL-1 cell
A punctate nuclear staining in untreated BCBL-1 cells was considered positive for antibodies to LANA. The viral lytic cycle was induced by incubating BCBL-1 cells with 20 ng/ml of 12-O-tetradecanoylphorbol-13-acetate (TPA; Sigma, St. Louis, MO, USA) for 96 h. Entire cell fluorescence in about 20% of TPA-treated cells was considered positive for antibodies to the lytic-phase antigens.

In individuals from the general population, seropositivity for HHV-8 antibodies against lytic or latent antigens ranged from 1.0 to 4.1% in different age groups. In the group of patients at risk for sexually transmitted diseases, the prevalence rates for HHV-8 antibodies were strikingly higher, ranging from 32.6% in the MSM group to 39.2% in AIDS patients without KS and 98.7% in AIDS patients with KS (Table 1).

Our data suggest that HHV-8 has a restricted circulation in children and young adults from the general population of São Paulo State and a high prevalence in individuals at risk for sexually transmitted diseases. This pattern of seroprevalence parallels the pattern observed in the United States, northern Europe and Japan.

The interpretation of HHV-8 seroepidemiologic studies has been hampered by the lack of a gold standard certifying the absence of infection. On the other hand, the sensitivity of HHV-8 serologic assays is limited by the fact that only KS patients are true positives (17). It is now recognized that antibody titers are higher in KS patients when compared with non-KS HHV-8-infected persons (18), and higher antibody titers are easier to detect. Therefore, the inclusion of KS patients as reference only in calibration groups to establish the sensitivity of a test may yield good performances even though tests may differ in sensitivity.

However, even in KS patients with a diagnosis confirmed by histopathological examination, the anti-LANA IFA has shown a lower sensitivity when compared with the lytic IFA (16). For this reason, using the less sensitive LANA IFA in non-KS HHV-8-infected individuals may underestimate the seroprevalence of HHV-8 infection in these populations (17).

As expected, the lytic IFA showed a higher sensitivity than the LANA IFA for detection of HHV-8 antibodies in the general population. The uncertain accuracy in asymptomatic HHV-8 infection is probably due to the low HHV-8 antibody titers in this group when compared with patients with KS/HIV. Nevertheless, even when subjects who were

<table>
<thead>
<tr>
<th>Age group</th>
<th>N</th>
<th>Lytic IFA</th>
<th>95% CI*</th>
<th>LANA IFA</th>
<th>95% CI</th>
<th>Lytic or LANA +</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-11 months</td>
<td>197</td>
<td>8 (4.1%)</td>
<td>1.9-8.1</td>
<td>0</td>
<td>-</td>
<td>8 (4.1%)</td>
<td>1.9-8.1</td>
</tr>
<tr>
<td>12-23 months</td>
<td>177</td>
<td>3 (1.7%)</td>
<td>0.4-5.3</td>
<td>0</td>
<td>-</td>
<td>3 (1.7%)</td>
<td>0.4-5.3</td>
</tr>
<tr>
<td>2-10 years</td>
<td>98</td>
<td>1 (1.0%)</td>
<td>0.1-6.4</td>
<td>0</td>
<td>-</td>
<td>1 (1.0%)</td>
<td>0.1-6.4</td>
</tr>
<tr>
<td>11-17 years</td>
<td>56</td>
<td>1 (1.8%)</td>
<td>0.1-10.8</td>
<td>0</td>
<td>-</td>
<td>1 (1.8%)</td>
<td>0.1-10.8</td>
</tr>
<tr>
<td>18-30 years</td>
<td>115</td>
<td>3 (2.6%)</td>
<td>0.7-8.0</td>
<td>0</td>
<td>-</td>
<td>3 (2.6%)</td>
<td>0.7-8.0</td>
</tr>
<tr>
<td>Total</td>
<td>643</td>
<td>16 (2.5%)</td>
<td>1.5-4.1</td>
<td>0</td>
<td>-</td>
<td>16 (2.5%)</td>
<td>1.5-4.1</td>
</tr>
<tr>
<td>High risk group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSM</td>
<td>95</td>
<td>20 (21.1%)</td>
<td>12.6-30.9</td>
<td>19 (20.0%)</td>
<td>12.8-29.7</td>
<td>31 (32.6%)</td>
<td>23.6-43.1</td>
</tr>
<tr>
<td>Non-KS HIV+</td>
<td>130</td>
<td>45 (34.6%)</td>
<td>26.6-43.5</td>
<td>32 (24.6%)</td>
<td>17.7-33.1</td>
<td>51 (39.2%)</td>
<td>30.9-48.2</td>
</tr>
<tr>
<td>KS AIDS patients</td>
<td>78</td>
<td>76 (97.4%)</td>
<td>90.2-100.0</td>
<td>59 (75.8%)</td>
<td>64.4-84.3</td>
<td>77 (98.7%)</td>
<td>92.1-99.9</td>
</tr>
</tbody>
</table>

*Fleiss quadratic 95% confidence interval (95%CI) calculated using Epi6 software. IFA = immunofluorescence assay; KS = Kaposi’s sarcoma; LANA = latency-associated nuclear antigen; Lytic = lytic-phase antigens; MSM = men who have sex with men.
reactive to either of the two IFA were classified as being infected with HHV-8, the prevalence of HHV-8 infection in children and adults in the present series was low.

In a previous study, a blinded comparison of seven immunofluorescence assays carried out at five experienced laboratories has shown that current HHV-8 antibody tests show a better interassay correlation in KS patients and in non-KS HIV-positive patients compared with healthy blood donors (19). In addition, a recent study has shown that titers to both lytic and latent antibodies increased for many years, usually in parallel, in longitudinally followed homosexual men (20). These findings probably explain the better agreement among lytic and LANA IFA in the group of patients at high risk for HHV-8 infection (MSM and AIDS patients with or without KS).

The prevalence of antibodies against this virus varies widely between different countries or within different geographical regions in the same country (11). Brazil is a large country, with a multiple ethnic and cultural composition. Therefore, the data obtained in the present study, relatively limited both in terms of sample number and geographical distribution, cannot be extrapolated to the Brazilian population at large.

References

17. Martin JN, Amad Z, Cossen C, Lam PK, Kedes DH, Page-Shafer KA, Osmond DH & Forghani B (2000). Use of epidemiologically well-defined subjects and existing immunofluorescence assays to cali-

