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

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Abstract

Human herpesvirus 8 (HHV-8) is a newly described herpesvirus that is etiologically associated with all forms of Kaposi’s sarcoma (KS). Seroepidemiological 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
- Seroeprevalence
- AIDS
- Kaposi’s sarcoma
- Immunofluorescence assay
- Latency-associated nuclear antigen
- Lytic-phase antigens

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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. Seroprevalence 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, Jaboticabal, 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 seroconversion 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
line. 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 consid-
ered positive for antibodies to the lytic-phase
antigens.

In individuals from the general popula-
tion, 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 trans-
mitted 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 re-
stricted circulation in children and young
adults from the general population of São
Paulo State and a high prevalence in indi-
ciduals at risk for sexually transmitted
diseases. This pattern of seroprevalence par-
allels the pattern observed in the United States,
northern Europe, and Japan.

The interpretation of HHV-8 seroepide-
miologic studies has been hampered by the
lack of a gold standard certifying the ab-
sence of infection. On the other hand, the
sensitivity of HHV-8 serologic assays is lim-
ited by the fact that only KS patients are true
positives (17). It is now recognized that anti-
body titers are higher in KS patients when
compared with non-KS HHV-8-infected per-
sons (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 popula-
tion. The uncertain accuracy in asympto-
matic 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>0-11 months</td>
<td>197</td>
<td>8 (4.1%)</td>
<td>1.9-9.1</td>
<td>0</td>
<td></td>
<td>0</td>
<td></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>0.7</td>
<td></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>1.0</td>
<td></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>1.0</td>
<td></td>
</tr>
<tr>
<td>16-50 years</td>
<td>115</td>
<td>3 (2.6%)</td>
<td>0.7-8.0</td>
<td>0</td>
<td></td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>448</td>
<td>16 (2.5%)</td>
<td>1.5-4.1</td>
<td>0</td>
<td></td>
<td>16.1</td>
<td></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>13.6-30.9</td>
<td>12.8-29.7</td>
<td>31 (32.6%)</td>
<td>19 (20.0%)</td>
<td></td>
</tr>
<tr>
<td>Non-KS HIV+</td>
<td>130</td>
<td>45 (34.6%)</td>
<td>26.4-53.5</td>
<td>22 (24.6%)</td>
<td>17.7-33.1</td>
<td>51 (39.2%)</td>
<td></td>
</tr>
<tr>
<td>KS AIDS patients</td>
<td>76</td>
<td>76 (57.4%)</td>
<td>50.2-99.0</td>
<td>59 (75.6%)</td>
<td>64.4-84.3</td>
<td>77 (96.7%)</td>
<td></td>
</tr>
</tbody>
</table>

*Fleiss quadratic 95% confidence interval; 95% CI calculated using Ep6 software; IFA = immunofluores-
cence 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

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