High Seroprevalence of Antibodies to Human Herpesvirus-8 in Egyptian Children: Evidence of Nonsexual Transmission

Massimo Andreoni, Gamal El-Sawaf, Giovanni Rezza, Barbara Ensoli, Emanuele Nicastrì, Laura Ventura, Lucia Ercoli, Loredana Sarmati, Giovanni Rocchi

Background: In western countries, human herpesvirus-8 (HHV-8) appears to be transmitted mainly by sexual contact. To evaluate the role of other transmission routes, especially in developing countries, we estimated the seroprevalence of HHV-8 in Egyptian children, who, if seropositive, would have acquired the virus through a nonsexual route.

Methods: Sera from 196 children (<1–12 years of age), 20 adolescents (13–20 years of age), and 30 young adults (21–25 years of age) attending a vaccination program in Alexandria, Egypt, were studied. Immunofluorescence assays were used to detect antibodies against HHV-8 lytic-phase antigens (anti-lytic) and latent-phase antigens (anti-latent). Antibodies against Epstein-Barr virus viral capsid antigen, cytomegalovirus, and HHV-6 were detected by enzyme-linked immunosorbent assays. Seroprevalence of these herpesviruses was calculated after stratifying the subjects by age.

Results: Anti-lytic and anti-latent HHV-8 antibodies were detected in 44.7% and 8.5% of the study participants, respectively. The prevalence of anti-lytic antibodies tended to increase with age, exceeding 50% in children older than 6 years; once children reached the age of 10 years, the prevalence tended to stabilize. The seroprevalence of other herpesviruses tended to be higher than that of HHV-8, ranging from approximately 83% to more than 97% in the 9- to 12-year age group. One- to 3-year-old children had higher titers of anti-lytic HHV-8 antibodies than children in the other age groups. Anti-latent antibodies were more frequently detected in individuals with high anti-lytic antibody titers.

Conclusions: HHV-8 antibodies are highly prevalent in Egyptian children, suggesting that, in developing countries, HHV-8 infection may be acquired early in life through routes other than sexual transmission. The lower seroprevalence of HHV-8 relative to that of the other herpesviruses suggests that HHV-8 is less transmissible than other common herpesviruses.

[A novel human herpesvirus, referred to as Kaposi’s sarcoma (KS)-associated herpesvirus/human herpesvirus-8 (HHV-8), has been identified recently in KS lesions (1). The detection of HHV-8 DNA sequences in virtually all patients with KS, with or without human immunodeficiency virus (HIV) infection, has allowed researchers to hypothesize a causal role for this virus in the pathogenesis of KS (2–6). The association between HHV-8 and KS has been confirmed by cross-sectional (7–10) and longitudinal (11–14) studies. In addition, the virus has also been consistently detected in primary-effusion B-cell lymphomas (15) and in Castleman’s disease (16).

Among HIV-infected individuals, homosexual men appear to be at higher risk of HHV-8 infection than intravenous drug users, individuals with hemophilia, and women (8,9). These data and the detection of HHV-8 DNA in semen (17) suggest that the virus may be sexually transmitted.

The extent to which HHV-8 is a ubiquitous infectious agent is still unclear. It is not known whether HHV-8 may also be transmitted by routes similar to those of other herpesviruses and whether the mode of transmission may differ according to geographic and socioeconomic settings. Studies of blood donors have shown little or no infection among U.S. blood donors and have shown intermediate and high prevalence rates among Italian blood donors and Ugandan blood donors, respectively.

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tively (7). Studies of children conducted in the United States have failed to detect HHV-8 infection, as defined by viral sequences in the peripheral blood mononuclear cells (18), or have shown a low prevalence or the absence of anti-lytic-phase antibodies with no anti-latent-phase antibodies (9,10,18). In contrast, HHV-8 DNA sequences have been detected in a high proportion of Japanese children (19). To our knowledge, limited data are available for children from developing countries (20), where the prevalence of HHV-8 in adults is high (7,9).

In industrialized western countries, the findings reported above suggest that HHV-8 is mainly transmitted through sexual intercourse, where most infections have been detected in homosexual men; yet these findings also suggest that other routes of transmission may play a role, especially in developing countries, where the infection appears to be widespread.

To test the hypothesis that HHV-8 infection may be acquired largely before an individual reaches a sexually active age, we evaluated the prevalence of anti-lytic and anti-latent HHV-8 antibodies in Egyptian children. To better evaluate the epidemiologic pattern, we also studied the seroprevalence of HHV-8 and other herpesviruses that are known to be widespread and are usually acquired early in life in developing countries.

MATERIALS AND METHODS

Study Participants

Serum samples were taken from 246 apparently healthy individuals recruited at the Medical Research Institute of the University of Alexandria, Egypt, where they were attending a vaccination program. Consecutive children, adolescents, and young adults (<25 years old) attending the vaccination program were recruited until approximately 40 individuals were recruited for each age group was reached. Demographic and clinical information on the participants was recorded on standardized forms. All participants were HIV seronegative.

Serologic Assays

Antibodies to lytic and latent antigens of HHV-8 were detected by using two different immunofluorescence assays (IFAs) based on the BCBL-1 (body cavity B-cell lymphomas) cell line (obtained through the acquired immunodeficiency syndrome [AIDS] Research and Reference Reagent Program, Division of AIDS, National Institutes of Health). The BCBL-1 cells were grown in RPMI-1640 medium with 10% heat-inactivated fetal calf serum, antibiotics (penicillin at 100 U/mL and streptomycin at 100 μg/mL), and 5 × 10−6 M 2-mercaptoethanol.

For the IFA to anti-lytic antigens, BCBL-1 cells were treated for 48 hours with phosphorib 12-myristate 13-acetate at 20 ng/mL. Ten microliters of a cell suspension (4 × 10^5 cells/mL) was smeared on slides, air-dried at room temperature, and then fixed in methanol/acetic acid, 1:1 (vol/vol), at −20°C for 10 minutes.

An IFA for anti-latent antigens was performed on isolated nuclei of BCBL-1 cells. Briefly, cells were washed twice with phosphate-buffered saline (PBS), resuspended in 10 mL of buffer containing 5% citric acid, and layered on 15 mL of 0.88 M sucrose in 5% citric acid. Nuclei were pelleted by centrifugation at 5000g for 5 minutes at 4°C, washed with PBS until the pH of the suspension was 7.4, and resuspended in PBS containing 0.25% fetal calf serum to a concentration of 1.5 × 10^6 nuclei/mL. Twelve microliters of the suspension was then smeared on slides, air-dried at room temperature, and fixed in methanol/acetic acid at −20°C for 10 minutes. For IFA, fixed smears were preblocked by incubation with PBS containing 3% fetal calf serum for 30 minutes in a humidified chamber and then incubated sequentially for a 45-minute period at 37°C with the test serum diluted 1:10 (in PBS containing 1% glycine and 2% fetal calf serum) and for a 45-minute period at 37°C with fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin antibodies. Titrations were done by 1:4 serial dilutions. An inverse titer of 10 or more was considered positive. All microscopic examinations were evaluated by investigators blinded to the status of the specimens.

Quantitative detection of antibodies to Epstein-Barr virus (EBV) viral cap antigen, cytomegalovirus (CMV), and HHV-6 were done by immunoenzymatic assays (viral capsid antigen immunoglobulin G [VCA IgG], CMV immunoglobulin G [IgG], and HHV-6 immunoglobulin G [IgG]) by the Diagnostic Products Corporation, Los Angeles, CA.

Italian blood donors and patients with KS were included as reference groups.

Data Analysis

The prevalence of antibodies directed against herpesviruses was calculated after stratifying by age (<1, 1–3, 4–6, 7–9, 10–12, and >12 years). The participants were also stratified by anti-lytic HHV-8 antibody titers (<1:10 or negative, 1:10, 1:50–1:200, and >1:400 dilution) and by age. The odds ratio (95% confidence intervals) of having anti-latent HHV-8 antibodies for individuals with different anti-lytic titers was also calculated.

RESULTS

Two hundred forty-six individuals were studied. Of those, 126 (51.2%) individuals were males and 196 (79.7%) individuals were less than 13 years old. Among the remaining 50 participants, 20 were between 13 and 20 years old and 30 were between 21 and 25 years old. The prevalence of antibodies to HHV-8 (anti-lytic and anti-latent), EBV viral cap antigen, CMV, and HHV-6, stratified by age, is shown in Table 1. Overall, 44.7% of the study population had anti-lytic antibodies against HHV-8. The highest relative increase in the proportion of children with anti-lytic HHV-8 antibodies was observed in the age group of 1–3 years, with a 20% increase compared with the age group of less than 1 year. The prevalence appeared to stabilize for children who were more than 9 years old. Of the 246 study participants, 8.5% had anti-latent antibodies; all participants with anti-latent antibodies also had anti-lytic antibodies. The prevalence of anti-latent antibodies was nearly stable across the age groups. Overall, HHV-8 seroprevalence was lower than that of the other herpesviruses.

The lower prevalence of anti-latent antibodies compared with the prevalence of anti-lytic antibodies that was found in the population studied may be attributed to a lower sensitivity of the anti-latent assay and/or, at least in part, to nonspecific cross-reactivity of the anti-lytic assay. This was addressed by including Italian normal blood donors and patients with KS as reference groups in the study. The

### Table 1. Prevalence of antibodies (immunoglobulin G) directed against Epstein-Barr virus (EBV) viral capsid antigen, cytomegalovirus, human herpesvirus-6 (HHV-6), and HHV-8 anti-lytic and anti-latent antigens according to the age group

<table>
<thead>
<tr>
<th>Age group, y</th>
<th>No. of subjects</th>
<th>Sex, male/female ratio</th>
<th>EBV</th>
<th>CMV</th>
<th>HHV-6</th>
<th>HHV-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>42</td>
<td>1.1 : 1</td>
<td>23</td>
<td>25</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>1–3</td>
<td>10</td>
<td>0.9 : 1</td>
<td>28</td>
<td>30</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>4–6</td>
<td>40</td>
<td>1.0 : 1</td>
<td>31</td>
<td>33</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>7–9</td>
<td>38</td>
<td>1.1 : 1</td>
<td>33</td>
<td>36</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>10–12</td>
<td>36</td>
<td>0.8 : 1</td>
<td>30</td>
<td>35</td>
<td>31</td>
<td>19</td>
</tr>
<tr>
<td>&gt;12†</td>
<td>50</td>
<td>1.4 : 1</td>
<td>44</td>
<td>50</td>
<td>42</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>246</td>
<td>1.1 : 1</td>
<td>189</td>
<td>209</td>
<td>160</td>
<td>110</td>
</tr>
</tbody>
</table>

*Lytic and latent refer to virus infection phases; anti-lytic and anti-latent antibodies are directed against viral antigens expressed predominantly in the corresponding phases.

†Including 20 adolescents between 13 and 20 years of age and 30 young adults between 21 and 25 years of age.

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the predominantly in cells in which HHV-8 is actively replicating.

In the study population, anti-HHV-6 antibodies were largely detected in the first year of life; then an almost 50% decrease in the seroprevalence was observed, with a symmetric dramatic increase after the children reached 10 years of age. Anti-CMV and anti-EBV antibodies had a similar pattern of increase; at least 70% of the children were exposed to these viruses within their 3rd year of life, and seroprevalence for CMV approached 100% in late childhood.

The distribution of anti-lytic antibody titers to HHV-8 by age group is reported in Table 2. The proportion of children with high titers (>1:400 dilution) of antilytic antibodies was higher in the age group of 1–3 years compared with older individuals. Participants with high antilytic titers were more likely to have antialt antibodies than those with low intermediate titers. Antialt antibodies were detected in 33.3% (seven of 21) of children with high titers and in 15.1% (eight of 53) and 16.7% (six of 36) of those with intermediate (1:50–1:200 dilution) and low (1:10 dilution) titers, respectively. Although the risk of having antialt antibodies was 2.68 times higher among those participants with high antilytic titers than among the other HHV-8-positive participants, the difference was not statistically significant (95% confidence interval = 0.81–8.83).

### Discussion

The results of our study suggest that HHV-8 infection is widespread among Egyptian children. The anti-lytic antibody prevalence was more than 16% in children less than 1 year of age and more than 50% among the group of 7- to 9-year-old children. The prevalence of antialt antibodies ranged from 5% to 10%, without a clear trend of increase over the years. The ratio between antialt and antialt antibodies was 5.2:1, ranging from 2.3:1 in the first age group to 9.5:1 in 9- to 12-year-old age group. Anti-lat antibodies were more commonly detected in children with high antilytic titers, but no difference was observed between those with low titers (1:10 dilution) and those with intermediate titers (1:50–1:200 dilution). The difference between antilytic and antialt seroprevalence may suggest that antialt antibodies are generally present at higher levels than antialt antibodies, although the data may reflect, at least in part, a low sensitivity of the antialt assay or a low specificity of the antilytic assay. It is interesting to note, however, that under the most stringent conditions for the antilytic assay (dilution >1:400), the seroprevalence was similar to that of the antialt assay. In addition, the prevalence of antialt antibodies in Italian blood donors (8%) was comparable to the prevalence of antialt antibodies found in blood donors from different regions of Italy (7%–20%).

The variable magnitude of the increase in HHV-8 seroprevalence suggests that most primary infections occur during the second year of life. This finding is consistent with the high proportion of 1- to 3-year-old children with high antibody titers, which may be representative of a recent infection. Anti-lytic antibody prevalence tends to stabilize after children reach the age of 10 years. However, due to the small number of participants who were more than 12 years old, no conclusions about the increase of HHV-8 seroprevalence in adolescents and young adults can be made.

The Egyptian pattern appears to differ from that determined in studies conducted in the United States that showed a low prevalence of antialt antibodies and the absence of either antialt antibodies to HHV-8 or anti-lytic and antialt antibodies and DNA sequences in children. The difference observed in the results of studies carried out in children from Egypt and the United States can be compared with serologic studies among adults, reporting higher prevalences of HHV-8 in some developing areas, such as Central Africa compared with some developed areas of the world, where prevalence of antialt antibodies among 142 Italian blood donors was 7.7% and 3.5%, respectively. Antilytic antibodies were detected in 100% of 49 patients with KS from Italy (35 with AIDS-related KS and 14 with classic KS); antialt antibodies were detected in 57.1% of the patients with AIDS-related KS and in 92.9% with classic KS. The results indicate, therefore, that cross-reactivity of the antilytic assay is negligible under the conditions used, although the higher prevalence of antilytic antibodies (compared with antialt antibodies) in serum from Italian patients with acquired immunodeficiency syndrome and KS is consistent with a lower sensitivity of the antialt assay. The specificity of the antilytic assay is further supported by our recent results showing that the presence of antialt antibodies in Italian individuals at risk of KS is highly predictive of disease development and, further, that the risk of KS development is associated with antialt antibody titers (Rizza G, Andreoni M, Dorrucci M, Pezzotti P, Monini P, Zerboni R, et al.: submitted for publication).

Table 2. Distribution of titers of antilytic* human herpesvirus-8 (HHV-8) antibodies by age group

<table>
<thead>
<tr>
<th>Age group, y</th>
<th>No. of subjects</th>
<th>No. (%) of subjects with antilytic HHV-8 antibody titers†</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>42</td>
<td>1 (2.3) 4 (9.5) 2 (4.7) 35 (83.3)</td>
</tr>
<tr>
<td>1–3</td>
<td>40</td>
<td>8 (20) 6 (15) 1 (2.5) 25 (62.5)</td>
</tr>
<tr>
<td>4–6</td>
<td>40</td>
<td>3 (7.5) 11 (27.5) 4 (10) 22 (55)</td>
</tr>
<tr>
<td>7–9</td>
<td>38</td>
<td>3 (7.9) 13 (34.2) 6 (15.7) 16 (42.1)</td>
</tr>
<tr>
<td>10–12</td>
<td>36</td>
<td>3 (8.3) 11 (30.5) 5 (13.8) 17 (47.2)</td>
</tr>
<tr>
<td>&gt;12‡</td>
<td>50</td>
<td>3 (6) 8 (16) 18 (36) 21 (42)</td>
</tr>
<tr>
<td>Total</td>
<td>246</td>
<td>21 (8.5) 53 (21.5) 36 (14.6) 136 (55.2)</td>
</tr>
</tbody>
</table>

*Antilytic refers to a virus infection phase; antialt antibodies are directed against viral antigens expressed predominantly in cells in which HHV-8 is actively replicating.

†Presented in terms of antibody dilutions for the assay; the greater the dilution, the higher the titer (i.e., the >1:400 dilution category indicates the highest titer).

‡Including 20 adolescents between 13 and 20 years of age and 30 young adults between 21 and 25 years of age.
a small proportion of blood donors, or none at all, was found to be seropositive (7,9,10).

With regard to routes of transmission, findings (8,9,14) by others suggest that in industrialized countries, HHV-8 infection is likely to be acquired after adolescence, during the sexually active phase of life, and it is mainly transmitted through sexual intercourse. Our findings suggest that in developing countries, the infection appears to be acquired early in life, as a possible consequence of transmission in the family and in community settings. It has been recently reported that infectious virus and viral RNAs may be detected in the saliva of patients without KS (23), and it has been suggested that HHV-8, like EBV, undergoes lytic replication in oropharyngeal cells (24). However, it may be possible that HHV-8 is less transmissible through the saliva than other herpesviruses, such as EBV and CMV; for this reason, nonsexual transmission may play an important role only when particular environmental conditions prevail (i.e., in the presence of overcrowding and poor hygiene and primarily in areas where the background HHV-8 prevalence in the general population is high). It is thus possible that the impact of specific transmission modalities may vary across different geocultural and socioeconomic contexts.

Comparison with other widespread herpesviruses may provide additional support for these hypotheses. In Egypt, the existence of environmental conditions favoring the spread of infections requiring nonsexual intimate contact is confirmed by a seroepidemiologic pattern that is typical of underdeveloped and tropical areas (25) and population groups of low socioeconomic level (26), characterized by a high prevalence of viral capsid antigen antibodies in early childhood. At the same time, HHV-8 seroprevalence appears to be lower than that of other herpesviruses that are mainly transmitted through saliva, such as HHV-6, EBV, and CMV.

Another aspect of our study that merits consideration is the relationship between HHV-8 and KS. If an association, suggested by longitudinal studies, is assumed, we would expect that, in a determined geographic area or population group, a higher KS incidence would reflect a higher HHV-8 seroprevalence. To date, the paradigm of this ecologic association has been confirmed by studies showing a parallel increasing level of HHV-8 prevalence and KS incidence from the United States to Mediterranean countries, such as Italy, to sub-Saharan Africa (7). In contrast, Egypt appears to be a country with a low incidence of KS. KS represents less than 1% of all the cancers diagnosed in the country and a minority of all skin tumors (27). Thus, the high prevalence of HHV-8 in Egyptian children is not consistent with the low incidence of KS, suggesting that other cofactors play an important role in the pathogenesis of KS in persons who have no known causes of immunosuppression, such as HIV infection or organ transplants. The hypothesis of different virus variants with different pathogenic spectrums should also be investigated (28). However, the possibility that KS incidence in Egypt is underestimated cannot be ruled out, and studies conducted among Egyptian transplant recipients suggest that the relative frequency of KS is slightly higher than in other international series (29). For this reason, caution is needed in interpreting the apparent disconnection between the presence of HHV-8 and KS rates.

In conclusion, our data show that HHV-8 infection is widespread among Egyptian children, suggesting that sexual activity is not the only route of transmission in developing countries. The high prevalence of individuals exposed to the virus is in apparent contrast with the low incidence of KS reported in Egypt. Other cofactors might be involved in the pathogenesis of KS in HIV-negative individuals.

**REFERENCES**


NOTES

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