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Kaposi’s Sarcoma Associated with Previous Human Herpesvirus 8 Infection in Kidney Transplant Recipients

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This study investigates the prevalence of human herpesvirus 8 (HHV-8) infection in kidney transplant patients, evaluating the risk of HHV-8 transmission via transplantation and the association between pre- and posttransplantation HHV-8 infection and the subsequent development of Kaposi’s sarcoma (KS). Immunofluorescence and an enzyme immunoassay were used to determine HHV-8 seroprevalence in 175 patients awaiting kidney transplantation and 215 controls who were attending our clinic for other reasons. All patients in the study came from central or southern Italy. Seroprevalence was similar in both groups (14.8 versus 14.9%), with no significant difference between the rates for male and female patients. Of the 175 patients, 100 were tested for anti-HHV-8 antibodies at various times during follow-up. During follow-up, seroprevalence increased from 12% on the date of transplantation to 26%. This increase was paralleled by an age-related increase in seroprevalence in the control group. During follow-up from 3 months to 10 years after transplantation, KS was diagnosed in seven patients (4.0%). Six of these patients were positive for HHV-8 prior to transplantation. Overall, 23.0% of patients who were HHV-8 positive before transplantation developed KS, whereas only 0.7% of seronegative patients developed the disease (relative risk, 34.4; 95% confidence interval, 4.31 to 274.0). This finding suggests that the key risk factor for KS is infection prior to transplantation and that antibody detection in patients awaiting transplantation could be useful in identifying patients at high risk for KS. In patients from geographic areas with a high prevalence of HHV-8, serological tests on donors may be less important.

Human herpesvirus 8 (HHV-8; also known as Kaposi’s sarcoma-associated herpesvirus) has been associated with all forms of Kaposi’s sarcoma (KS), including transplantation-associated KS. Although its role in pathogenesis has yet to be defined, HHV-8 has been identified as an important cofactor in the development of KS. HHV-8 has been detected in virtually all KS patients. Viral DNA and serum antibodies to HHV-8 appear to have predictive value for the onset of KS, especially in patients with compromised immune systems (2, 5, 7, 20).

HHV-8 is not, however, restricted to KS patients. Seroprevalence in the general population varies. Higher-than-average seroprevalence has been reported in geographic areas such as central and southern Italy, where classical KS is also more frequent than in other parts of the world. A strict association between HHV-8 and KS has been demonstrated in transplant recipients. HHV-8 seroprevalence and iatrogenic KS also appear to be correlated (6, 9, 11, 15). It is still unclear whether posttransplantation KS is due to the reactivation of HHV-8 as a result of immunosuppressive treatment or to primary HHV-8 infection transmitted via organ transplantation (16, 18, 19).

The aim of this study was to compare HHV-8 seroprevalence in a group of patients awaiting kidney transplants and in a control group. All patients in the study came from central or southern Italy. Transplant recipients were studied during follow-up in order to evaluate the risk of HHV-8 transmission via kidney transplantation and the correlation between HHV-8 infection and the subsequent development of KS.

**MATERIALS AND METHODS**

**Patients and specimens.** Serum samples were collected from 175 out of 212 patients who underwent transplantation in the Transplant Unit of the Università Cattolica del Sacro Cuore Rome, Rome, Italy. Data collection took place over a 9-year period from 1989 to 1997. Patients whose sera prior to transplantation were not available and patients lost at follow-up were excluded from the study. In this period the waiting list for our Transplantation Unit included over 600 patients. The low percentage of transplants is a consequence of a scarcity of donors in central and southern Italy. The mean age of transplant patients (TP) was 39.9 years (range, 16 to 52), with a male/female ratio of 1.6:1.

Patients were monitored by physicians from the Transplantation Unit for up to 10 years after transplantation. Follow-up visits were scheduled every 3 months during the first year after transplantation and every 6 months subsequently. Diagnoses of KS were made by the Transplantation Unit’s resident dermatologist, using physical examination and routine laboratory tests (complete blood cell count, lymphocyte subsets in the peripheral blood, and blood chemistry profile). Clinical diagnoses were confirmed by histological examination and HHV-8 DNA detection in lesional skin and in peripheral blood. Where clinically warranted, chest X-rays, abdominal ultrasound scans, gastrointestinal tract endoscopy, and computerized axial tomography scans were performed.

The 175 TP enrolled in our study were compared with 215 controls randomly selected from patients undergoing routine examinations at the Outpatient Department at the Institute of Microbiology at the Università Cattolica del Sacro Cuore. The mean age of the controls was 45.2 years (range, 18 to 69 years), with a male/female ratio of 1:1. All patients (TP and controls) came from central and southern Italy. More than 60% lived in the Lazio region.

During follow-up, serum samples were collected from 100 out of the 175...
kidney transplant recipients who gave their informed consent. Data on blood transfusions and years under dialysis were also collected.

**HHV-8 antibody detection.** Serum was tested using an immunofluorescence assay based on the HHV-8-infected cell line BC-3 (American Type Culture Collection, Manassas, Va.). Serum was stimulated with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA; Sigma, St. Louis, Mo.) to induce the lytic cycle, as described elsewhere (6). Cells were spotted onto welled slides, fixed in cold acetone for 10 min, and incubated with 20 µl of human serum that was diluted 1:2, starting from 1:80, until no reactivity was observed. Slides were then washed, incubated with a prestandardized dilution of Kallestad fluorescein-conjugated goat F(ab')2, fragment anti-human immunoglobulin G (Sanofi Diagnostics Pasteur, Chaska, Minn.), washed again, air dried, and examined under a fluorescence microscope. Samples showing specific reactivity at a 1:80 dilution were considered positive for HHV-8 antibodies. Positive results for reactive samples were confirmed by immunoenzymatic assay (HHV-8 IgG antibody; Advanced Biotechnologies, Columbia, Md.) as recommended by the supplier.

**RESULTS**

All serum samples from TP and controls were tested for HHV-8 antibodies (Table 1). No significant difference was detected between pretransplant seroprevalence in TP (14.8%) and seroprevalence in controls (14.9%). In neither group was there any significant difference in rates for males and females. In the control group, seroprevalence tended to increase with age, with a rate of 10.8% in patients between 18 and 49 years old (121 of 215) and 20.3% in patients over 50 (94 of 215). Antibody titers (ranging from 1:80 to 1:2,560) were similar for both groups of patients. The staining patterns observed were specific for reactivity with lytic viral antigens. Samples tested with only secondary antibody showed no reactivity.

Follow-up testing of 100 out of the initial 175 TP, in the period from 3 months to 10 years after transplantation, showed an increase in seroprevalence from 12 to 26%. During this period, 14 (16%) of the 88 patients who had tested negative for HHV-8 on the day of transplantation seroconverted; the remaining 74 patients continued to test negative.

In our group of TP we observed seven cases of iatrogenic KS after kidney transplantation. This relatively high rate could be explained by the fact that our patients came from central and southern Italy, which have a higher-than-average rate of HHV-8 infection and where classical KS is endemic (1, 6, 23). This hypothesis is supported by previous reports of a strong correlation between KS and HHV-8 infection (5, 7, 17).

DISCUSSION

Prevalence rates and transmission mechanisms for HHV-8 are still under study. There is, however, widespread agreement that rates of HHV-8 prevalence are relatively higher in Mediterranean and African areas and lower in Western countries (6, 9, 11, 15).

In this study, the overall rate of HHV-8 seroprevalence in patients awaiting transplant was 14.8%. This rate was statistically indistinguishable from the rate (14.9%) observed in controls. The observed rate of seropositivity in pretransplant patients was higher than rates reported in other European countries (8, 19) and can be explained by the higher-than-average HHV-8 seroprevalence in the general population of central and southern Italy (5, 14, 22, 23). The data suggest that HHV-8 seroprevalence is unaffected by pretransplant therapeutic regimens. This conclusion is supported by other studies showing a low rate of parenteral transmission of HHV-8 (8, 12, 13). The lack of any statistically significant association between HHV-8 seropositivity and blood transfusion or years of dialysis suggests that HHV-8 transmission may involve other routes (4, 5, 6, 10, 15, 22).

In this study, 7 (4.0%) out of 175 patients developed iatrogenic KS after kidney transplantation. Six of seven KS patients were seropositive prior to transplantation, and only one was seronegative. Of the seropositive patients, 23.0% developed KS, as opposed to 0.7% of patients who tested negative for HHV-8 (relative risk, 34.4; 95% CI, 4.31 to 274.0). This result shows a significantly increased risk of KS in patients who are seropositive prior to transplantation.

The observed 12 to 26% increase in rates of seropositivity following transplantation is paralleled by the age-related increase in prevalence observed in the control group and by previous reports pointing in the same direction (14, 15). This suggests that seroconversion due to transplantation may be

<table>
<thead>
<tr>
<th>Patients</th>
<th>Total no.</th>
<th>Mean age (yr)</th>
<th>Male/female ratio</th>
<th>No. (%) HHV-8 seropositive</th>
<th>No. (%) HHV-8 seronegative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transplant recipients (pretransplant)</td>
<td>175</td>
<td>39.9</td>
<td>1.6</td>
<td>26 (14.8)</td>
<td>149 (85.2)</td>
</tr>
<tr>
<td>Controls</td>
<td>215</td>
<td>45.2</td>
<td>1.1</td>
<td>32 (14.9)</td>
<td>183 (85.1)</td>
</tr>
</tbody>
</table>

TABLE 2. KS in kidney transplant recipients and presence of serum antibodies against HHV-8 before transplantation

<table>
<thead>
<tr>
<th>HHV-8 seropositivity before transplantation</th>
<th>No. of patients</th>
<th>% Incidence of KS (no. of cases)</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHV-8 negative</td>
<td>149</td>
<td>0.7 (1)</td>
<td>34.4 (4.31–274.0)</td>
</tr>
<tr>
<td>HHV-8 positive</td>
<td>26</td>
<td>23.1 (6)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>175</td>
<td>4.0 (7)</td>
<td></td>
</tr>
</tbody>
</table>
relatively rare. Of the 14 patients who seroconverted, only 2 seroconversions were observed within 6 months of transplantation; in such instances it is impossible to exclude donor transmission. Neither of these patients developed KS during 3 years of follow-up.

The only case of KS among the 14 seroconverted patients was observed 30 months after transplantation; unfortunately, it was not possible to determine the exact time at which seroconversion had occurred. Of the seven patients who developed KS, this patient was the only one who presented a complete remission of the disease (cutaneous and gastric KS) after withdrawal of treatment with cyclosporine and methylprednisolone. This suggests an important role for immunosuppression.

In this study we did not have access to donor data. We therefore cannot exclude the possibility that in some cases seropositive organ recipients were reinfected with HHV-8 from donors. The data nonetheless suggest that pretransplant HHV-8 seropositivity is a more important risk factor for KS than infection via transplantation.

We suggest that in geographic areas where HHV-8 infection is frequent and many patients are seropositive before transplantation, immunosuppressive treatment may induce the reactivation of latent infections, playing an important role in the development of iatrogenic KS in association with virus- or host-associated factors (16). This of course does not exclude the possibility that, in populations with a low prevalence of HHV-8, primary HHV-8 infection acquired through or after transplantation may play a role in iatrogenic KS, with immunosuppressive therapy acting as the most important cofactor.

In conclusion, our study suggests that antibody detection in transplant recipients could be useful to detect patients with high risk for KS and that, at least in areas where HHV-8 infection is common, this may be more important than HHV-8 antibody detection in donors.

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