HERPES VIRUS INFECTION PREVALENCE IN REGULAR HAEMODIALYSIS PATIENTS – A COMPARATIVE EVALUATION OF COMPLEMENT FIXATION, INDIRECT IMMUNOFLUORESCENCE AND ELISA TESTS

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Summary
The presence and titres of specific serum IgG and IgM antibodies to cytomegalovirus, herpes simplex virus and varicella-zoster virus were evaluated in 50 haemodialysis patients by complement fixation, immunofluorescence and Elisa tests. A second serum sample was tested in 24 patients after four weeks. Specific serum IgG antibodies to Epstein-Barr virus were also measured by immunofluorescence in 26 patients.

By immunofluorescence and Elisa tests, the prevalence of cytomegalovirus, herpes simplex virus and Epstein-Barr virus infection is approximately 100 per cent, and varicella-zoster virus 60 per cent. High titres of IgG specific antibodies found by Elisa tests, detection of IgM antibodies in 16–18 per cent of patients and sero-conversion in 25 per cent of patients, suggests continuous antigenic stimulation.

Introduction
Herpes virus family includes a group of agents (cytomegalovirus, herpes simplex virus, varicella-zoster virus, Epstein-Barr virus) known for their widespread distribution in humans and in numerous other mammals. Herpes viruses are able to persist in infected cells in a latent form and can be reactivated, commonly in immunosuppressed patients. The high rate of herpes virus infection in recipients of renal allografts is well known, occurring in up to 90 per cent of patients [1,2].

In contrast, the frequency and importance of herpes virus infection in haemodialysis patients is incompletely understood, though immunological surveys of chronic renal failure patients and transplant candidates reveal a prevalence of antibodies to cytomegalovirus, herpes simplex virus, varicella-zoster virus and Epstein-Barr virus ranging from 54 to 100 per cent [3–6]. Moreover, unexplained febrile illnesses, pericarditis, pneumonitis and HBs-Ag negative hepatitis have been described in association with a significant rise in antibody titres to cytomegalovirus and Epstein-Barr virus [7–9].
To investigate the prevalence and role of herpes virus infection we conducted a serological survey of haemodialysis patients in our centre.

Patients and methods

The study group included 50 haemodialysis patients (37 males, 13 females) with an average age of 44 years (range 22–78). The average period of haemodialysis treatment was four years (range 1–12).

Serum specimens were drawn from all patients and tested for cytomegalovirus, herpes simplex virus, varicella-zoster virus, IgG antibodies by complement fixation, immunofluorescence and Elisa tests. Sera were absorbed with Rheumatoid Factor Latex suspension, before testing for IgM antibodies to cytomegalovirus, herpes simplex virus, varicella-zoster virus by immunofluorescence and Elisa tests. A second serum sample was examined for cytomegalovirus, herpes simplex virus, varicella-zoster virus, IgG antibodies in 24 patients four weeks later. Specific serum IgG antibodies to Epstein-Barr virus were also measured by immunofluorescence in 26 patients.

Titres equal to or greater than 1:4 by complement fixation, 1.8 by immunofluorescence, 1:640 by Elisa were considered positive (inactive infection). Seroconversion (active infection) was diagnosed on the basis of a four-fold or greater increase in antibody titres by complement fixation, immunofluorescence and Elisa tests.

Results

The percentages of patients with detectable IgG antibodies, the range of antibody titres and geometric mean titres for cytomegalovirus, herpes simplex

<table>
<thead>
<tr>
<th></th>
<th>% Seropositive patients</th>
<th>Range of Ab titres</th>
<th>GMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CMV</td>
<td>CF 30%</td>
<td>4–64</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>IF 96%</td>
<td>8–1024</td>
<td>161</td>
</tr>
<tr>
<td></td>
<td>EL 88%</td>
<td>640–20480</td>
<td>2238</td>
</tr>
<tr>
<td>2. HSV</td>
<td>CF 76%</td>
<td>8–64</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>IF 100%</td>
<td>64–4096</td>
<td>446</td>
</tr>
<tr>
<td></td>
<td>EL 94%</td>
<td>1280–4096</td>
<td>4160</td>
</tr>
<tr>
<td>3. VZV</td>
<td>CF 6%</td>
<td>4–16</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>IF 60%</td>
<td>8–256</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>EL 56%</td>
<td>640–5120</td>
<td>1795</td>
</tr>
<tr>
<td>4. EBV</td>
<td>IF 92%</td>
<td>16–512</td>
<td>90</td>
</tr>
</tbody>
</table>

Ab = antibodies; GMT = geometric mean titres; CMV = cytomegalovirus; HSV = herpes simplex virus; VZV = varicella-zoster virus; EBV = Epstein-Barr virus; CF = complement fixation; IF = immunofluorescence; EL = Elisa
virus, varicella-zoster virus and Epstein-Barr virus, obtained by each test are shown in Table I.

Quantifiable IgM antibodies to cytomegalovirus were detected in five (10%) patients by immunofluorescence and Elisa; IgM antibodies to herpes simplex virus in two (4%) by immunofluorescence and in three (6%) patients by Elisa. One patient (2%) showed both anti-cytomegalovirus and anti-herpes simplex virus IgM antibodies. IgM and anti-varicella-zoster virus antibodies were not found.

Seroconversion was ascertained in three (12%) patients for cytomegalovirus, two (8%) for herpes simplex virus, one (4%) for varicella-zoster virus. Antibody changes were simultaneous and of the same order of magnitude by complement fixation, immunofluorescence and Elisa tests. All patients with active infection were asymptomatic.

Discussion

This study confirms, as observed in other surveys, the high prevalence of herpes virus infections in haemodialysis patients [3–6]. By more sensitive tests, Elisa and immunofluorescence, inactive infection was diagnosed in 88–96 per cent of patients for cytomegalovirus, 94–100 per cent for herpes simplex virus, 56–60 per cent for varicella-zoster virus and 92 per cent for Epstein-Barr virus. Complement fixation test showed lower sensitivity and failed to detect an appreciable number of sera with antibody detectable by Elisa and immunofluorescence.

High anti-cytomegalovirus and anti-herpes simplex virus titres were obtained by Elisa. In animal models, it has been found that persistent antigenic stimulation is necessary for maintenance of high antibody titres to herpes simplex virus [10]. The high antibody titres detected in our patients may actually be a reflection of antigenic load at the time of the study.

IgM anti-cytomegalovirus and/or anti-herpes simplex virus antibodies were detected in 16–18 per cent of patients. Generally, measurable titres of IgM antibodies indicate active or recent immunological stimulus by a specific agent and offer an indication of recent infection. Active infection, defined as significant increase in antibody titres, was detected in 12 per cent of patients for cyto-

megalovirus, eight per cent for herpes simplex virus and four per cent for varicella-zoster virus. No overt infectious disease was found in these patients.

Our data suggest a continuous antigenic stimulation by herpes virus in our haemodialysis patients. Active infection from herpes virus after renal transplantation almost always seems to be a secondary infection. Prospective serological and epidemiological investigations should be undertaken to determine the extent to which herpes viruses contribute to pneumonitis, pericarditis, unexplained febrile illnesses and Hbs Ag negative hepatitis among haemodialysis patients.

References


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