Detection of Human Herpesvirus 6 and Varicella-Zoster Virus in Tear Fluid of Patients with Bell’s Palsy by PCR

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Received 13 December 1999/Returned for modification 18 February 2000/Accepted 28 April 2000

Human herpesvirus 6 DNA was detected by PCR in the tear fluid of 7 (35%) of 20 patients with Bell's palsy and of 1 (5%) of 20 healthy controls. Varicella-zoster virus was detected by PCR in the tear fluid of 2 of 20 Bell's palsy patients but in none of the tear fluids from 20 healthy controls. These findings suggest an association between human herpesviruses and Bell's palsy.

Much attention has been paid to the possibility that Bell's palsy has a herperviral etiology, and the most clearly defined viral cause of facial palsy is varicella-zoster virus (VZV) infection, known as the Ramsay-Hunt syndrome. Recent evidence suggests that herpes simplex virus type 1 (HSV-1) may be a major etiologic agent in Bell's palsy (6). In the mentioned study, however, HSV-1 was detected in the endoneural fluid collected during decompression surgery from the facial nerve of Bell's palsy patients (6). Notably, after primary infection, HSV, which is often latent in the geniculate ganglion, may be reactivated in the ganglia after surgical stress (7). Human herpesvirus 6 (HHV-6) is a common neurotropic virus which has been associated with conditions such as febrile convulsions, encephalitis, and multiple sclerosis (5) and is thus another candidate in Bell's palsy. The etiology of Bell's palsy is difficult to study directly, since it is almost impossible to obtain samples through noninvasive procedures. One possibility is to test saliva. However, HHV-6 itself replicates in the salivary glands and, like HSV-1, is commonly detected in the saliva of healthy persons (3, 5). This led us to investigate other possibilities. The facial nerve not only carries nerve impulses to the muscles of the face and to the salivary glands but also through the nervus intermedius, arising from the geniculate ganglion, secretory motor fibers to the lacrimal gland. Therefore, we searched for human herpesviruses HHV-6, VZV, HSV-1, and HSV-2 by PCR from the tear fluid samples of Bell's palsy patients to determine the possible association of herpesviruses in Bell's palsy.

Bilateral tear fluid samples were obtained at the time of the first visit from 20 patients (aged 21 to 74 years; median age, 44 years) with a sudden, isolated, peripheral facial palsy of an unknown etiology at the Department of Otorhinolaryngology of Helsinki University Central Hospital from September 1998 to March 1999. Twenty healthy age- and sex-matched persons (aged 21 to 56 years; median age, 39 years) from the staff of the clinic served as controls. The controls had no history of Bell's palsy or other related syndromes or neurological diseases. The local ethics committee approved the study, and informed consent was obtained from all patients and controls. All tear fluid samples were collected with a scaled 25-μl fire-polished microcapillary tube by holding its tip at 10 to 30° over the horizontal axis and at 10 to 40° to the surface of the lower fornix (11). The

samples were immediately transferred to sterile microtubes and stored at −70°C until assayed. Case histories were recorded; none of the Bell's palsy patients or the controls had any other known herperviral infections. All patients were investigated in the acute phase, 1 to 7 days after the onset of facial palsy (median, day 2). Thereafter, the patients underwent clinical examination every 3 months until recovery. No medical or other treatment modalities, except lubrication drops for dry eyes, were used. Electroneuronography (ENOG) and pure-tone audiometry were done on all of the patients. Antibodies against Borrelia burgdorferi flagellin were measured from all of the Bell's palsy patients. Two clinically diagnosed HSV keratitis patients and one VZV blepharitis patient served as positive controls. The keratitis patients' tear fluid samples were collected from the affected eye only.

DNA was isolated from 15 μl of tear fluid by proteinase K digestion followed by phenol extraction and ethanol precipitation. Two different PCR programs were used for amplification of specific parts of HSV, VZV, and HHV-6. The amplification procedure for HSV and VZV consisted of 40 cycles at 96°C for 10 s, 55°C for 20 s, and 72°C for 20 s, and the procedure for HHV-6 consisted of 40 cycles at 94°C for 30 s, 51°C for 1 min, and 72°C for 1 min. Both programs also consisted of a denaturation step at the beginning (at 95°C for 10 to 13 min), and for both programs the final step was allowed to continue for 5 min. The primers used in PCRs were chosen from the polymerase genes for HSV-1 and HSV-2 as recommended by Piiparinen and Vaheri (8) (5'-biotin-AAG GAG GCG CCC AAG CTG CCG-3' and 5'-TGG GTG ACT ACA GCC ACG TGG CAA AGT-3'), for VZV as recommended by Echevarria et al. (2) (5'-AGG TAC CAT GAA AAG CTT GCC TCC TCG-3' and 5'-AGT GCC GAG ACC GCT ATT AAT C-3'), and from the U67 gene for HHV-6 as recommended by Gopal et al. (4) (5'-AGG TCG TGG TCC TCG GGG TCC TGG TCC-3' and 5'-TTG GGT ACA GGC TGG CAA AGT-3'), for HHV-6 as recommended by Gopal et al. (4) (5'-AGG TAC CAT GAA AAG CTT GCC TCC TCG-3' and 5'-AGT GCC GAG ACC GCT ATT AAT C-3'). Positive and negative controls were included in each run. In addition, an internal standard was used for every sample to detect inhibitory samples. The standard included the sites for HHV-6 primers, and the sequence of the amplified fragment was randomized. The amplified products were detected by microplate hybridization.

Hybridization was carried out as described for HSV using luminometric reading (12). Briefly, PCR products were immobilized to streptavidin-coated microplates and double-stranded products were denatured by alkali. For hybridization specific digoxigenin-labeled oligonucleotide probes were used (5'-CCC TCC TCG CTT TCG TCC TCG-3' for HSV-1, 5'-TCC TCG...
HC:**AC TGT CTG ACT GGC AAA AAC TTT T-3

**CGC TAC CGG AAC GTA TGC CAC AAG-3**

**TCG TCG TCC TTA ATC C-3**

TGGC TGATGGC AAC GTA TGC CAC AAG-3

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strate (Lumigen, Southfield, Mich.). The sample was consid-
ered positive if the relative light unit signal was >7-fold higher
than the background signal. Altogether 7 out of 82 tear fluid
samples (9%) were found to be inhibitory.

HHV-6 DNA was detected by PCR from tear fluid samples
in a total of 7 (35%) Bell's palsy patients and in 1 (5%) healthy
control (P = 0.044; Fisher's Exact Test) at the time of the
sampling. Altogether HHV-6 DNA was detected in 10 out of
the total of 40 tear fluid samples (25%) collected from the
patients and in 1 (3%) out of 39 tear fluid samples collected
from the healthy controls. HHV-6 was found in two tear fluid
samples from the same side as the facial palsy, and in four
cases HHV-6 was found in the tear fluid of the contralateral
eye (Table 1). At the time of the Bell's palsy diagnosis, ENOG
showed no response in three herpesvirus-positive patients
(38%), <50% response in three herpesvirus-positive patients
(38%), and 50 to 90% response in two herpesvirus-positive
patients (25%) (Table 1). Of 12 herpesvirus-negative Bell's
palsy patients ENOG showed no response in one patient (8%),
<50% response in one patient (8%) and 50-90% response in
ten patients (84%). In one case the ENOG response was also
worse on the opposite side, in one case the eye on the paretic
side was so dry that it was difficult to obtain a tear fluid sample,
and in one case VZV was also found from bilateral tear fluid
samples. VZV was found in four tear fluid samples from two
Bell's palsy patients (10%) (Table 1). One herpesvirus-nega-
tive patient had symptoms suggestive of upper respiratory tract
infection within 2 weeks prior to the onset of Bell's palsy. Four
patients (one HHV-6 positive, one VZV positive, and two
herpesvirus negative) had diabetes which was controlled by
medication. Pure-tone audiometry was normal or showed no
acute changes in all of the patients. Antibodies against B.
burgdorferi were not found in any of the patients. Neither
HSV-1 nor HSV-2 was found in the tear fluid samples of Bell's
palsy patients or of the healthy controls. HSV-1 DNA was
found in two tear fluid samples of two clinically diagnosed
HSV keratitis patients, and VZV DNA was found in one tear
fluid sample of one clinically diagnosed VZV blepharitis pa-
tient.

In the present study we found that HHV-6 and VZV were
detectable by PCR in the tear fluid samples of Bell's palsy
patients. Among 20 Bell's palsy patients there were two pa-
tients whose facial paralysis did not recover during the 1-year
follow-up period. The tears of one patient were PCR positive
for HHV-6, and the tears of the other patient were PCR
positive for VZV. Developments in antiviral treatments may
offer better therapeutic measures for early treatment of viral
Bell's palsy. Of seven HHV-6-positive Bell's palsy patients, one
was also positive for VZV. Whether this coinfection of her-
pesviruses in Bell's palsy influences the course of the disease
is not known. A limited number of studies have demonstrated
coinfection of the central nervous system with herpesviruses.
One study showed herpesvirus coinfection with HSV and
HHV-6 by PCR in 7% of patients with central nervous system
disease (9). Among 20 Bell's palsy patients we found two pa-
tients positive for VZV. This result confirms earlier studies
in which VZV reactivation without cutaneous vesicles has been
demonstrated in up to 25% of cases (1, 10). Recently, VZV
DNA has been detected by PCR in oropharyngeal swabs from
patients with acute peripheral facial paralysis without zoster
lesions in the oral cavity and skin (3).

In conclusion, our results show that HHV-6 DNA can be
detected in the tear fluid of a significant number of Bell's palsy
patients. Whether this virus plays a role in the etiopathogenesis
of Bell's palsy remains to be determined. It is noteworthy that
we found VZV DNA in tear fluid samples from two Bell's palsy
patients, showing that VZV can be detected in the tear fluid of
patients with Bell's palsy without cutaneous vesicles.

Riitta Heino, Leena Palmunen, Kaisa Aaltonen, and Teija Tekkala
are acknowledged for their expert technical assistance.

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**TABLE 1. Detection of virus by PCR using the tear fluid samples of Bell's palsy patients**

<table>
<thead>
<tr>
<th>Patient gender</th>
<th>Patient age (yr)</th>
<th>Duration of symptoms (day)</th>
<th>Duration of recovery</th>
<th>Paresis side</th>
<th>HHV-6 detection of*</th>
<th>% ENOG response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>22</td>
<td>1</td>
<td>10 days</td>
<td>Left</td>
<td>Right</td>
<td>Negative 90%</td>
</tr>
<tr>
<td>Male</td>
<td>23</td>
<td>1</td>
<td>10 days</td>
<td>Left</td>
<td>Left</td>
<td>Negative 40</td>
</tr>
<tr>
<td>Male</td>
<td>45</td>
<td>7</td>
<td>No recovery</td>
<td>Left</td>
<td>Left and right</td>
<td>Negative 40</td>
</tr>
<tr>
<td>Male</td>
<td>47</td>
<td>4</td>
<td>9 months</td>
<td>Left</td>
<td>Negative</td>
<td>No response</td>
</tr>
<tr>
<td>Male</td>
<td>58</td>
<td>1</td>
<td>6 months</td>
<td>Left</td>
<td>Left</td>
<td>Negative 80</td>
</tr>
<tr>
<td>Male</td>
<td>60</td>
<td>1</td>
<td>6 months</td>
<td>Right</td>
<td>Left and right</td>
<td>Negative 40</td>
</tr>
<tr>
<td>Male</td>
<td>71</td>
<td>4</td>
<td>No recovery</td>
<td>Right</td>
<td>Negative</td>
<td>No response</td>
</tr>
<tr>
<td>Male</td>
<td>73</td>
<td>3</td>
<td>10 days</td>
<td>Left</td>
<td>Right</td>
<td>Negative 90%</td>
</tr>
</tbody>
</table>

* ENOG response from the right side.

b The tear fluid sample was difficult to get from the left eye because of the dryness of the eye.

c Results are given as the eye(s) whose tear sample(s) was PCR positive for the indicated virus.

