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HHV-6-positivity in diseases with demyelination

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ABSTRACT

Background: The triggering agent of multiple sclerosis is still unknown and many viruses, including human herpesvirus-6 (HHV-6), are under suspicion. In earlier study we found patients who had HHV-6 reactive OCBs in their CSF. We wanted to investigate whether HHV-6 has an active role in diseases with demyelination.
Objective: To analyze the HHV-6-reactive cases in detail and investigate the possible independent role of HHV-6 in the development of central nervous system involvements with demyelination.
Study design: We studied serum and CSF samples that were collected over a period of one year, from all patients who had oligoclonal bands (OCB) in cerebrospinal fluid (CSF) and were examined in the Department of Neurology, University Central Hospital of Helsinki, Finland. Clinical evaluation was accomplished blinded of HHV-6 analysis and follow-up time was two years. All patients underwent MRI of the head and clinically indicated CSF analysis.
Results: The 17 patients with HHV-6-reactive OCBs were significantly younger and had significantly more IgG-OCBs in comparison to patients without HHV-6-reactive OCBs. Initial diagnoses in patients with HHV-6-reactive OCBs remained the same during the follow-up time.
Conclusion: Patients with HHV-6-positive OCBs appear to form a separable group. In progressive neurological diseases HHV-6 may have a role in long-term infection with demyelination.

1. Background

Diseases with central nervous system (CNS) demyelination can cause severe motoric, sensory, and cognitive deficiencies for the patients. The most known demyelinating disease, that causes severe disability in young adults, is multiple sclerosis (MS) [1]. The etiology is still mostly unknown but many viruses are under suspicion as a possible triggering agent including human herpesvirus-6 (HHV-6) [2].

HHV-6 is a highly neurotropic virus that has tropism to many cell types of neural origin. Type 6A seems to be more neurovirulent than 6B [3]. Besides its neurotropic character, HHV-6 has tropism for a variety of other human cells and tissues. It is capable of infecting different organs like lungs and liver [4,5]. After primary infection, HHV-6 enters into host mononuclear cells in various organs, especially in salivary glands [6,7]. This latency, together with neurotropism, may be the key to inducing neurological diseases [8].

In the cerebrospinal fluid (CSF) there are IgG-class antibodies that B-cells produce [9]. Using isoelectric focusing (IEF), these intrathecal antibodies form visible separate bands called oligoclonal bands (OCB). OCBs specific to CNS should by definition be found in CSF with no counterparts in serum, though in some cases weak serum counterparts may be visible. The role of OCBs in neurological diseases remains poorly understood. The presence of OCBs is one part of McDonald's MS-criteria [10–12]. Among patients with MS, OCBs are present in CSF in more than 95% of cases [13]. The presence of OCBs, however, is not a specific laboratory parameter for any specific neurological disease. OCBs occur in patients with CNS infections and are present in inflammation and in neurodegenerative diseases [14–16]. OCBs are reactive for the causative agent in herpes simplex encephalitis and in chronic measles infections [17–19].

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Table 1
Results of patients with HHV-6 positive findings in more detail. Some patients had only HHV-6 serological primary infection, some had only reactive OCBs, and some had both.

Patient number	No. of HHV-6-reactive OCBs, type 6A	No. of HHV-6-reactive OCBs, type 6B	(No. of HHV-6-reactive OCBs)/(No. of total OCBs) × 100%	Serological primary infection	Serological past infection	Diagnosis at 2 years	Diagnosis at 0 year	MRI (Barkhof *x*/4)
1	4	3	33	No	No	MS	MS	4
2	0	1	4	No	6A + 6B	MS	MS	0
3	1	5	24	No	6A + 6B	CIS	CIS	3
4	2	0	12	No	6A + 6B	CIS	CIS	3
5	3	0	13	No	6A + 6B	MS	MS	4
6	5	4	75	No	6A + 6B	MS	MS	3
7	5	4	53	No	6A + 6B	Parestesia	Parestesia	0
8	4	4	42	No	6A + 6B	MS	MS	4
9	0	2	11	No	6B	CIS	CIS	0
10	2	1	12	No	6A + 6B	MS	MS	3
11	1	0	3	No	6A + 6B	MS	MS	3
12	2	1	13	No	6A	MS	MS	4
13	0	2	11	No	6A + 6B	CIS	CIS	1
14	1	3	25	No	6A + 6B	MS	MS	3
15	6	0	35	6A	6B	ADEM	ADEM	4
16	0	2	7	6A + 6B	No	MS	MS	0
17	0	1	5	6A + 6B	No	MS	MS	4
18	0	0	0	6A	6B	MS	MS	3
19	0	0	0	6B	6A	MS	MS	4
20	0	0	0	6A + 6B	No	Mollaret's meningitis		0
21	0	0	0	6A	6B	Epilepsy	Epilepsy	0
22	0	0	0	6B	6A	CIS	Mb n.peronei	0
23	0	0	0	6A + 6B	No	MS	MS	4
24	0	0	0	6A	6B	MS	MS	2
25	0	0	0	6A	6B	MS	MS	4
26	0	0	0	6B	6A	MS	MS	4
27	0	0	0	6B	6A	CIS	CIS	2
28	0	0	0	6B	6A	CIS	CIS	2
29	0	0	0	6A + 6B	No	RA	MS	0
30	0	0	0	6B	6A	MS	MS	3

MS, multiple sclerosis; CIS, clinically isolated syndrome; ADEM, acute disseminated encephalomyelitis; RA, rheumatoid arthritis.

2. Objectives

In an earlier study, we presented OCBs reactivity for HHV-6 [20]. Now we have studied the relevance of this finding further.

3. Study design

3.1. Patients and sample collection

All patients who had neurological symptoms and who clinicians had remitted for further neurological studies to the Department of Neurology, University Central Hospital of Helsinki, Finland, and had positive result in OCB detection were included in the study. The collection of serum and CSF samples of those patients continued a period of one year. Seventy-nine patient's samples were available for further retrospectively made analysis of OCBs. IEF, immunofixation, and affinity-driven immunoblot served to visualize reactive OCBs in CSF as described [20]. Seventeen patients had HHV-6-reactivity detected in the OCBs in CSF, and 62 had no HHV-6-reactivity in the OCBs (Table 1). A specialist in clinical chemistry re-evaluated all OCBs without knowing previous data, HHV-6 findings or clinical course. None of the patients have lately had corticosteroid-treatment before their spinal tap. The Ethics Committee of Helsinki University Central Hospital has approved the study.

3.2. Laboratory tests

3.2.1. Antibody test

Immunofluorescence antibody test (IFA) showed for analysis of the levels of HHV-6A and HHV-6B antibodies in serum and CSF, as described earlier [21].

3.2.2. Avidity test

We tested the avidity of antibodies with a urea wash, as described earlier [21]. IgG antibodies with high binding strength indicate serological past infection; antibodies with low binding strength a serological primary infection.

3.2.3. Other laboratory tests

We determined CSF parameters WBC and RBC, protein, albumin and IgG concentration, glucose level, IgG index (the ratio of IgG and albumin in CSF to the ratio in serum), lysozyme level, and in selected cases angiotensin-1-converting enzyme by standard methods of the clinical laboratory. We analyzed IgG and IgM antibodies for Borrelia burgdorferi from all serum and CSF samples by an enzyme-linked immunosorbent assay. We used a TPHA test to exclude *Treponema pallidum* infection.

3.3. Magnetic resonance imaging

A specialist in neuroradiology evaluated the every patient's brain MRI scans blindly using Barkhof's criteria [22].

3.4. Hospital records

A senior neurologist confirmed the neurological diagnosis on every patient without knowing the HHV-6 results. All the diagnoses of MS were based on McDonald's-criteria [10–12]. Clinical follow-up was continued for two years.