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Detection and Typing of Human Herpesvirus 6 by Molecular Methods in Specimens from Patients Diagnosed with Encephalitis or Meningitis▽

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Human herpesvirus 6 (HHV-6) was detected in specimens from patients hospitalized with symptoms of encephalitis or meningitis. A real-time PCR assay was developed which has a linear dynamic range of 5 to 5 × 10^6 copies of HHV-6 and a sensitivity of five gene copies per reaction. While the assay detects both subtypes, HHV-6A and HHV-6B, it is specific and does not cross-react with a selected specificity panel. A total of 1,482 patient specimens, which were collected between 2003 and 2007, were tested; 26 specimens from 24 patients were found to be positive for HHV-6 by real-time PCR. The HHV-6 detection rate in this population was therefore 1.75%. The majority of the specimens tested (>95%) were cerebrospinal fluid (CSF) specimens. We were able to type 20 of the 26 positive specimens by conventional PCR and sequence analysis; all were HHV-6B. Forty-two percent of the patients were 3 years of age or younger, which may indicate a primary infection in these patients. Given the ages of the remaining patients (from 4 to 81 years), their infections were most probably due to virus reactivations. Where information was available, symptoms of patients included fever (71%), altered mental status (67%), and abnormal CSF profile (75%). Fifty percent of patients of 3 years of age or younger suffered from seizures. The detection of HHV-6 in specimens from patients diagnosed with encephalitis or meningitis, in the absence of a positive PCR result for other agents, strongly suggests a role for HHV-6 in the pathogenesis of these central nervous system diseases.

Human herpesvirus 6 (HHV-6) belongs to the betaherpes virus subfamily of the family *Herpesviridae* (14). HHV-6 is one of the most prevalent herpesviruses in humans. There are two variants, HHV-6A and HHV-6B, which have an overall genetic identity of 90% (17). Clinical disease caused by HHV-6A is not well understood. HHV-6B causes exanthema subitum (roseola infantum) (45), febrile illness, febrile seizures, or fever with otitis media. Exanthema subitum, a childhood disease, is characterized by a high fever and the development of a rash after the fever has resolved. By the age of three, almost all children have been infected by HHV-6 (25, 39), resulting in the majority of adults being seropositive (31). After primary infection, HHV-6 persists in the salivary glands and remains latent in monocytes and macrophages. Immunosuppression in an individual can lead to reactivation of the virus and various complications, including encephalitis (21, 34, 40). HHV-6 has been associated with delayed engraftment, high-grade graft-versus-host disease, and lymphoproliferative disorders (29, 32, 37). There are several reports of HHV-6 involvement in immunocompetent patients with acute encephalitis (1, 27, 36, 41) and meningitis (24).

Our laboratory performs diagnostic testing of specimens from patients diagnosed with encephalitis or meningitis. We have developed a real-time PCR assay for the detection of HHV-6 in these specimens. Once the virus is detected, it is then typed using a previously published conventional PCR assay (28). The target for the real-time PCR assay is a portion of the U6 gene. The function of the U6 gene is not known; however, the gene shows 95.1% sequence identity between the 6A and 6B subtypes and is unique to HHV-6 (17). The primers and probe were selected in order to achieve amplification of both HHV-6A and -6B. One of two primer sets was used for typing, targeting either the U86 or the U95 gene (28); both are immediate early/regulatory genes. Once amplified, HHV-6A and -6B produce fragments of different sizes, thereby allowing the type to be determined. In addition, the sequence of the amplified product was determined in order to verify the subtype.

MATERIALS AND METHODS

Clinical specimens. A total of 1,482 specimens from hospitalized patients in New York State who had been diagnosed with encephalitis or meningitis were tested for HHV-6 by real-time PCR. The majority of specimens (>95%) were derived from cerebrospinal fluid (CSF), but other matrices, including brain tissue, serum, plasma, and liver tissue, were also tested. Specimens were collected between the years 2003 and 2007. The requirements for testing were that a minimum of 500 μl liquid or 2 mm^2 of solid organ specimen be collected, frozen immediately upon collection, and sent to the Viral Encephalitis Laboratory on dry ice.

Viruses and controls. HHV-6A (strain 350) was obtained from the NIH AIDS Research and Reference Reagent Program, and HHV-6B was obtained from the ATCC (Manassas, VA). The plasmid control for HHV-6 (pNT10) was constructed via conventional PCR performed with the real-time PCR primers for the HHV-6 assay (Table 1) and HHV-6B as the template, and the product was then cloned into the Topo II plasmid (Invitrogen, Carlsbad, CA).

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TABLE 1. Primers and probes used in our singleplex real-time PCR assays for the detection of HHV-6 and GFP

Primer or probe	Sequence
HHV-6 forward primer	5′ AAAATTTCTCACGCCGGTATTC 3′
HHV-6 reverse primer	5′ CCTGCAGACCGTTCGTCAA 3′
HHV-6 probe	6-FAM-TCGGTCGACTGCCCGCTACCA-TAMRA
GFP forward primer	5′ CACCCTCTCCACTGACAGAAAAT 3′
GFP reverse primer	5′ TTTCACTGGAGTTGTCCCAATTC 3′
GFP probe	6-FAM-TGTGCCCATTAACATCACCAT CTAATTCAACA-TAMRA

As an internal extraction control and in order to detect PCR inhibition, each clinical specimen was spiked during the lysis step of the extraction process with a known quantity of plasmid pTU65 (8), which contains the green fluorescent protein (GFP) gene. Plasmids pNT10 and pTU65 were quantified by the determination of their absorbance values at 260 nm. Genomic HHV-6 DNA was quantified by performing real-time PCR on serial dilutions of plasmid and genomic DNAs and by constructing a standard curve using serial dilutions of quantified plasmid DNA as standards.

Nucleic acid extraction. Nucleic acid was extracted from patient specimens by using the NucliSENS miniMAG or easyMAG system (bioMérieux, Durham, NC). Two hundred and fifty microliters of each specimen was added to 2 ml of lysis buffer. Five microliters of plasmid pTU65 (1,900 gene copies) was spiked into the lysed sample. Following its extraction with miniMAG or easyMAG, the nucleic acid was eluted in 50 or 55 μl of elution buffer, respectively.

Real-time PCR. Real-time PCR for the detection of HHV-6 was performed using primers and probe developed in-house (Table 1). The probe was labeled with the reporter 6-carboxyfluorescein (6-FAM) at the 5′ end and the quencher 6-carboxy-tetramethyl-rhodamine (TAMRA) at the 3′ end. Amplification was carried out in a 25-μl volume reaction mixture by using universal buffer (ABI, Foster City, CA), 1,100 nM each primer, and 200 nM probe. The reaction mixtures were incubated at 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. A separate real-time PCR for the detection of GFP was performed with the primers and probes listed in Table 1. Labeling of the GFP probe was the same as that for the HHV-6 probe. The reaction mixture consisted of universal buffer, 900 nM each forward and reverse primer, and 250 nM probe. The reaction conditions were as described above. PCRs were performed using an ABI 7900 or ABI 7500 instrument.

Conventional PCR. A previously reported PCR method for the typing (28) of HHV-6 was used essentially as described previously, with primer sets targeting the U86 or U95 gene. PCR products were analyzed by agarose gel electrophoresis and stained by ethidium bromide. The expected product sizes were 311 and 209 bp for HHV-6B and HHV-6A, respectively, when the U86 gene was targeted, and 342 and 264 bp, respectively, when the U95 gene was targeted. PCR products of the expected size were extracted from the gel and spun through an Ultrafree-DA filter device (Millipore, Billerica, MA). Sequencing reactions of the PCR products were performed using the same primers that were used for the PCRs. The primer concentrations were 3.2 pmol, and sequencing reactions were performed at the Wadsworth Center Molecular Genetics Core facility on an automated DNA sequencer model 3100 (Applied Biosystems, Foster City, CA).

Sequence analysis. The obtained sequences were subjected to a BLAST search against the GenBank database. A subtype was assigned to each sample based on the homology of our sequence data with published sequences in GenBank.

RESULTS

The real-time assay for the detection of HHV-6 was determined to have a linear range from 5×10^6 gene copies/reaction (gc/rx) to 5 gc/rx, as determined from performance of real-time PCR on serial dilutions of quantified HHV-6 DNA extracted from culture. The efficiency of the reaction was 100%, with an R^2 value of 0.99 and an m value (slope of standard curve) of −3.32. The limit of detection of the assay was 5 gc/rx, equating to 200 gc/ml of patient specimen. A specificity assay was performed, in which the reactivity of the HHV-6 assay was determined against genomic nucleic acid from the following viruses and bacteria: influenza A-H1, influenza A-H3, influenza B, rhinovirus, echovirus 9, echovirus 11, echovirus 30, Coxsackie A and B viruses, human coronavirus 229E, respiratory syncytial virus, severe acute respiratory syndrome (SARS) coronavirus, human metapneumovirus, adenovirus, varicella-zoster virus, herpes simplex viruses 1 and 2, cytomegalovirus, Epstein-Barr virus (EBV), group A streptococcus, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Neisseria subflava*, *Streptococcus sanguis*, and *Corynebacterium xerosis*. No cross-reactivity was observed between the primer and probe set and the organisms selected in the specificity panel, indicating that the assay is specific.

We performed a retrospective screen of archived specimens from patients who had been hospitalized with a diagnosis of encephalitis or meningitis. Fourteen CSF specimens were from those collected in 2003, 86 from those collected in 2004, and 277 from those collected in 2005 selected randomly. We also tested a total of 1,105 specimens that were received from 1 January 2006 to 31 July 2007. Twenty-six specimens (from 24 patients) of the total 1,482 specimens were positive for HHV-6 (Table 2). Of these specimens, 20 could be typed and all 20 were found to be HHV-6B. The remaining specimens could not be typed, either because of insufficient specimen for testing or because the typing assay was not as sensitive as our real-time PCR detection assay was. All positive specimens were from CSF, except for a sample of liver tissue and a serum specimen (Table 2). Dual infections were detected in three cases. These included two patients with dual infections of EBV and HHV-6 and one autopsy specimen in which both adenovirus and HHV-6 were detected. Apart from the dual infections, varicella-zoster virus, herpes simplex viruses 1 and 2, EBV, cytomegalovirus, adenovirus, and enterovirus were ruled out by PCR or real-time PCR for all specimens. In addition, arboviruses, including West Nile virus, Saint Louis encephalitis virus, California serogroup viruses, Cache Valley virus, and eastern equine encephalitis virus were ruled out for specimens that were received during the arbovirus season (1 May to 30 November). Bacterial culture results were negative for seven specimens and not reported for the remaining specimens. Immunosuppression was not reported for any of the patients found to be positive for HHV-6. However, patient 9 had undergone a blood transfusion 4 weeks prior to the onset of his symptoms, and therefore may have been immunocompromised.

Three specimens were received from one patient (patient 23 [Table 2]) during the course of her illness. Two CSF specimens and one serum specimen were submitted for this patient. The first CSF specimen was collected 16 days after onset of illness; by performing real-time PCR, we were able to determine that the specimen had a viral count of approximately 13,000 gc/ml. The serum was collected 27 days after onset and had a viral count of approximately 22,000 gc/ml. The second CSF was collected 34 days after onset, as the patient symptoms were resolving, and had a viral count of 6,600 gc/ml.

The time between the onset of disease and specimen collection was reported for 20 of the 24 patients who tested positive for HHV-6 (Table 2). For these patients, the time range was 0 to 34 days, with a mean of 8.5 days. Three of the positive

TABLE 2. Demographic, clinical, and laboratory information for the 24 HHV-6-positive patients[a]

Patient no.	Sample type	Patient		Days between onset and collection	Diagnosis	Symptom(s)	CSF information				HHV-6 real-time PCR C_T value	HHV-6 typing result	Typing primer used	Other agents detected
		Sex	Age (yr)				CSF abnormality	Protein level (mg/dl)	WBC count	% Lymphs				
1	CSF	M	12	10	Encephalitis	Fever, altered mental status, hallucinations	Yes	56	35	95	31.78	B	U86	
2	CSF	F	12	2	Encephalitis	Fever, altered mental status	No	33	4	Unknown	31.98	B	U86	
3	CSF	F	2	7	Encephalitis	Fever, altered mental status, seizures, muscle weakness	Unknown	Unknown	Unknown	Unknown	36.07	Untyped	U86	
4	CSF	M	45	Unknown	Encephalitis	Fever, headache, seizures, altered mental status, muscle weakness, muscle pain	Yes	Unknown	20	80	34.3	Untyped	U86	
5	CSF	M	2	1	Encephalitis	Fever, seizures, altered mental status	No	16	0	Unknown	39.3	B	U95	
6	CSF	F	1	0	Unknown	Fever, rash, altered mental status, increased AST/ALT	Yes	483	2	50	38.65	Untyped	U86	
7	CSF	M	2	0	Meningitis	Fever, altered mental status, cough	Unknown	28	Unknown	Unknown	36.5	B	U86	
8	CSF	F	30	31	Encephalitis; acute onset psychosis	Headache, rash, altered mental status, confusion, hyperactivity	Yes	13	10	100	32.29	B	U95	
9	CSF	M	8	Unknown	Encephalitis	Seizures, altered mental status, muscle weakness, stiff neck	Yes	35	58	90	37.58	B	U95	
10	CSF	M	38	14	Encephalitis	Fever, altered mental status, headache, stiff neck	Yes	Unknown	Unknown	Unknown	39.74	Untyped	U95	EBV+ve
11	CSF	F	1	Unknown	Encephalitis	Seizures	Unknown	Unknown	Unknown	Unknown	33.3	B	U95	
12	CSF/autopsy	M	2	Unknown	Encephalitis; meningitis	Seizures	Unknown	Unknown	Unknown	Unknown	38.47	B	U95	
13	CSF	M	17	1	Meningitis	Headache, stiff neck, muscle weakness, muscle pain	Yes	150	40	29	25.03	B	U95	
14	CSF	F	81	5	Encephalitis	Fever, headache, rash, altered mental status	Unknown	Unknown	Unknown	Unknown	33.56	Untyped	U95	
15	CSF	F	4	0	Encephalitis	Fever, seizures, altered mental status	No	25	2	0	34.75	B	U95	
16	CSF	F	8	3	Meningitis	Fever, headache, stiff neck, meningeal sign	Yes	66	228	58	39.92	B	U95	
17	Liver tissue	F	30	7	Unknown	Fever, rash, elevated LFT result, jaundice, pruritis	N/A	N/A	N/A	N/A	34	B	U95	
18	CSF	M	60	1	Meningoencephalitis	Fever, seizures, altered mental status	Yes	49	99	N/A	28.89	B	U95	
19	CSF	M	25	1	Meningitis	Muscle weakness	Yes	119	49	100	28.68	B	U95	
20	CSF/autopsy	F	1	Unknown	Unknown	Sickle cell disease, anemia, fever	Unknown	Unknown	Unknown	Unknown	37.96	B	U95	Adv+ve
21	CSF/autopsy	M	2	Unknown	Unknown	Fever, cough, bronchiolitis symptoms	Unknown	Unknown	Unknown	Unknown	37.35	B	U95	
22	CSF	M	71	8	Encephalitis	Fever, altered mental status	Yes	117	47	100	29.62	Untyped	U95	EBV+ve
23A	CSF	F	3	16	Encephalitis; cerebellitis	Muscle weakness, ataxia	Unknown	Unknown	Unknown	Unknown	32.2	B	U95	

23B	Serum		27		Unknown	N/A	N/A	N/A	N/A	30.7	B	U95
23C	CSF		34		Headache, altered mental status, vomiting, tremor	No	15	1	Unknown	36.09	B	U95
24	CSF	M	1	2	Encephalitis	Fever, muscle weakness, altered mental status, seizures, rash	Yes	288	5	65	32.9	B	U95

[a] F, female; M, male; AST/ALT, alanine aminotransferase/aspartate aminotransferase; LFT, liver function test; % Lymphs, number of lymphocytes as a percentage of total WBC count; N/A, not applicable; Adv, adenovirus. U86 and U95 are the genes targeted in the HHV-6 PCR typing assays. Three specimens were tested for patient 23 (A, B, and C).

specimens were autopsy specimens (patients 12, 20, and 21 [Table 2]), and no onset date was available.

The ages of the 24 patients who were positive for HHV-6 ranged from 1 year to 81 years (Table 2). Ten patients were 3 years of age or younger, six were between 4 and 20 years old, and eight were between 21 and 81 years old. Forty-two percent were therefore infants or very young children. Thirteen of 24 patients were male.

Fifteen patients had been diagnosed with encephalitis, three with meningitis, one with encephalitis or meningitis, and one with meningoencephalitis (Table 2). The diagnoses for the remaining four patients were unknown. Three of the CSF specimens were from autopsy cases, and the CSF profiles were not reported. An additional seven specimens did not have CSF profiles available. The CSF profiles were reported for 16 cases; of these, 12 profiles were abnormal (Table 2). An abnormal CSF profile is defined as having a higher than normal protein range (>45 mg/dl) and/or a higher than normal white blood cell (WBC) count (>5 WBC/mm^3). Eight patients had proteins in the higher than normal range (49 to 483 mg/dl), with a mean of 166 mg/dl. Nine patients had higher than normal WBC counts, ranging from 10 to 228 WBC/mm^3, with a mean of 65. Six patients had both abnormal protein levels and abnormal WBC counts, and of these patients, all except one had predominantly lymphocyte increases. Two patients had abnormal protein levels but normal WBC counts, and two patients had abnormal WBC counts (predominantly lymphocytes) but normal protein levels. For one patient, the WBC count was abnormal, but the protein level was not reported.

Fever was reported for 17 (71%) patients, altered mental status was reported for 16 (67%) patients, headache was reported for 7 (29%) patients, and seizure was reported for 8 (33%) patients (Table 2). Other symptoms that were reported included muscle weakness, muscle pain, rash, and stiff neck. These are general symptoms seen in patients suffering from encephalitis or meningitis. Altered mental status is often also reported for patients with encephalitis.

Following real-time PCR, the cycle threshold (C_T) value was recorded for each specimen extract (Table 2). The C_T is defined as the number of cycles required for the fluorescent signal to cross the threshold (exceed background level). The C_T values for the specimen extracts ranged from 25.03 to 39.92, indicating a substantial variation among the viral loads of the patients (Table 2). Although the detection assay was not performed as a quantitative assay, the C_T value from real-time PCR can be inversely correlated with the viral titer, with a low C_T value indicating a high viral titer.

DISCUSSION

The various methods used for the diagnosis of HHV-6 include PCR, serology, viral culture, in situ hybridization, and immunohistochemistry. Since HHV-6 has the ability to persist and establish latency, the problem arises that diagnostic tests must be able to distinguish active viral replication from latent infection. Reverse transcription-PCR assays have been developed to detect the presence of viral mRNA, which constitutes a marker for active infection (44). The detection of immunoglobulin M or immunoglobulin G antibodies to HHV-6 in serum or CSF does not discriminate between active infection

and latent/chronic persistent infection. The same is true for the detection of HHV-6 DNA by PCR in peripheral blood mononuclear cells (PBMCs), the site where the virus establishes latency. In these cases, HHV-6 DNA can be detected by PCR in PBMCs at low levels (9). Viral culture is a difficult and time-consuming process and is not routinely used in clinical laboratories to detect virus in CSF specimens. Immunohistochemistry, although a valuable tool, requires brain specimens that are highly invasive and not generally available. In recent years, the use of quantitative PCR for absolute quantitation of viral load has come into wider usage (2, 19, 26) since viral load is a preferred indicator of active infection.

In order to detect HHV-6 in specimens from patients diagnosed with encephalitis or meningitis, we developed a real-time PCR assay targeting a portion of the U6 gene of HHV-6. This real-time PCR assay was shown to be sensitive, specific, and reproducible. The assay detects both subtypes 6A and 6B, and the primers and probe do not cross-react with the specificity panel selected for the assay. It detects as few as five gene copies, and with a turnaround time of less than 5 h, it is also rapid. The assay has an internal control, which allows inefficient extraction or PCR inhibition to be detected. In our experience, PCR inhibition in CSF specimens is uncommon ($<1\%$).

A total of 1,482 specimens submitted between 2003 and 2007, the majority of which were CSF specimens, were screened for HHV-6 by using real-time PCR. Twenty-six of the specimens, corresponding to 1.75% of the total specimens, were positive for HHV-6 and were subsequently typed by conventional PCR. The typing assay primer sets target the U86 and U95 genes (28). These genes encode immediate early proteins that control gene expression. When the two open reading frames are compared between HHV-6A and -6B, they are found to have $<70\%$ identity and both genes are larger in HHV-6B (20, 28). The primers that we selected allow the differentiation of the two subtypes on the basis of the size of the PCR product obtained. In addition, the subtype was verified by sequence analysis. In all cases where we were able to obtain a typing result, the subtype was HHV-6B (20 of 26 specimens). HHV-6B is more prevalent than HHV-6A, although HHV-6A has been suggested to have greater neurotropism (5, 22). There are differing opinions as to whether subtype 6A or 6B more frequently infects the central nervous system (CNS) (16). We were unable to detect HHV-6A in any of the patients in our study, but its presence has been reported by other groups who have studied CNS infections (12, 22).

Acute primary infection is likely to have occurred for the 10 patients who were 3 years of age or younger. For the remaining patients, virus reactivation is more likely given that asymptomatic persistence of HHV-6, especially HHV-6B, is common in healthy adults (22). Alternatively, it is possible that in older patients who have previously not been infected by HHV-6, primary infection has a higher likelihood of leading to more serious disease. Further studies are needed to elucidate the outcomes of primary infection in adults.

Most patients who were positive for HHV-6 in this study suffered from fever (71%) and an altered mental status (67%). Seizures were reported for 9 of the 24 patients in whom HHV-6 was detected; 5 of these seizure patients were 3 years of age or younger ($\leq$3 age group). Therefore, 50% of the $\leq$3 age group in whom HHV-6 was detected suffered from seizures. Febrile seizures appear to be caused by primary HHV-6 infection in infants; one study showed the incidence to be 13% in the United States population (23).

An abnormal CSF profile was reported for 75% of the HHV-6-positive patients for whom the CSF profile was reported. In general, a viral infection of the CNS results in a normal glucose level ($>$45 mg/dl), a normal or increased protein level, and an increased WBC count, predominantly with an increased number of lymphocytes. Although there is general agreement between HHV-6 positivity in specimens and abnormality of the CSF, this is not always the case. Therefore, it would be imprudent to limit HHV-6 testing only to patients with abnormal CSF profiles.

The tropism and latent establishment of HHV-6 in tissues, including brain tissue, make it difficult to interpret the detection of HHV-6 genome in the CSF of patients diagnosed with encephalitis or meningitis, even in the absence of other etiologies. Since there have been reports of HHV-6 DNA being detected in CSF specimens of immunocompetent individuals without CNS disease (6, 22), it has been suggested that a positive PCR result obtained for a CSF specimen should be interpreted on the basis of the clinical situation and should be deemed important only when a positive result for other agents has not been obtained (16). However, the positive PCR results obtained for the 26 specimens in our study, taken together with the clinical syndrome of each of the 24 patients, and the negative results obtained for other herpesviruses, adenovirus, enterovirus, and arboviruses, strongly suggest that HHV-6 infection was associated with the patient's clinical disease.

Three of the total 1,482 patients tested were found to have dual infections with EBV or adenovirus. HHV-6 and EBV are frequently detected simultaneously (3) given that both viruses are prevalent in lymphoid tissues. It has been shown that HHV-6 infection can activate EBV replication from latency (18) and that the presence of EBV genome can make B cells more susceptible to HHV-6 infection in vitro (11).

Reactivation of HHV-6 occurs in immunocompromised individuals, especially patients with AIDS or those undergoing solid organ, stem cell, or bone marrow transplantation (7). Immunosuppression was not reported for any of the patients who were positive for HHV-6 in our study, except possibly for patient 9, who had undergone a blood transfusion.

The outcome for HHV-6-positive patients is generally favorable, with most patients recovering fully. However, mild to moderate neurological impairment as well as death due to complications of neurological disease has been reported (27, 35, 41). Three of the specimens that were positive for HHV-6 in our study were autopsy specimens from infants of $\leq$2 years of age. Adenovirus was also detected for patient 20; furthermore, this patient appeared to have an underlying disease (sickle cell anemia). Despite the underlying disease, a rule-out of viral agents of encephalitis was requested at autopsy. The symptoms for patient 21 were suggestive of a respiratory disease rather than encephalitis. The remaining autopsy specimen was from a patient (patient 12) who had been diagnosed with encephalitis, and no other agents were identified. In these cases, we cannot conclude that HHV-6 infection was the cause of death, but it remains a possibility.

HHV-6 DNA has been shown to integrate into the human

genome and can be transmitted from parent to child in an integrated form (13, 42). In addition, stem cell transplantation can lead to the transmission of an integrated HHV-6 from a donor to a recipient (10). A study in Japan estimated that HHV-6 integration is present in 0.21% of the study population (42). Therefore, when testing for HHV-6, it is necessary that we distinguish between individuals who have an integrated HHV-6 and those who have an active infection. The integration of HHV-6 in the genome leads to abnormally high levels of HHV-6 DNA being present in PBMCs and can be detected by fluorescent in situ hybridization analysis (33). In the present study, we did not determine whether any of the HHV-6-positive patients had the virus integrated in their chromosomes. Therefore, although integration is unlikely in all cases, we cannot rule it out. Experiments to determine viral DNA titers in CSF specimens from healthy individuals who have HHV-6 integrated in their chromosomes is warranted, since it would allow a background level to be established.

Real-time PCR, due to its quantitative properties, is used in clinical diagnostics to identify a target copy number that is related to a sample's viral load. Through such measurement of the viral load, it may be possible to differentiate latent from active infection and also to monitor patients during the course of illness (30, 38), especially to verify the efficacy of antiviral therapy.

There are currently no approved agents for the treatment of HHV-6 infection. However, a number of antiviral agents, including ganciclovir, foscarnet, and cidofovir, have been shown to be effective against HHV-6 in vitro (46). In transplant patients, the use of foscarnet or ganciclovir has been shown to decrease HHV-6 viral load (43). Ganciclovir was used successfully to decrease the viral load in an immunocompetent individual diagnosed with meningoencephalitis (4), and it was also shown to lead to the recovery of an immunocompetent patient with HHV-6 encephalomyelitis (15).

Our data and previously reported data show that HHV-6 should be included in the differential diagnosis of encephalitis/meningitis cases in immunocompetent as well as immunocompromised individuals. Although we did not perform HHV-6 serology, a combination of molecular detection and serology is ideal for study cases. Further investigations are needed to determine the relationship between the presence of the viral genome in patient's CSF specimens and the actual role of the virus in pathogenesis in encephalitis/meningitis cases. Early detection of actively replicating HHV-6 in patients with clinical signs of encephalitis is important so that antiviral therapy can be initiated in order to attempt to resolve the neurological symptoms.

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REFERENCES

1. **Ahtiluoto, S., L. Mannonen, A. Paetau, A. Vaheri, M. Koskiniemi, P. Rautiainen, and M. Muttilainen.** 2000. In situ detection of human herpesvirus 6 in brain tissue from fatal encephalitis. Pediatrics **105:**431–433.
2. **Aritaki, K., J. H. Ohyashiki, A. Suzuki, T. Ojima, K. Abe, N. Shimizu, K. Yamamoto, K. Ohyashiki, and A. Hoshika.** 2001. A rapid monitoring system of human herpesvirus reactivation by LightCycler in stem cell transplantation. Bone Marrow Transplant. **28:**975–980.
3. **Bertram, G., N. Dreiner, G. R. Krueger, A. Ramon, D. V. Ablashi, S. Z. Salahuddin, and N. Balachandram.** 1991. Frequent double infection with Epstein-Barr virus and human herpes-6 in patients with acute infectious mononucleosis. In Vivo **5:**271–279.
4. **Birnbaum, T., C. S. Padovan, B. Sporer, T. A. Rupprecht, H. Ausserer, G. Jaeger, and H. W. Pfister.** 2005. Severe meningoencephalitis by human herpesvirus 6 type B in an immunocompetent woman treated with ganciclovir. Clin. Infect. Dis. **40:**887–889.
5. **Boutolleau, D., C. Dura, P. Bonnafous, D. Caiola, A. Karras, N. De Castro, M. Ouachee, P. Narcy, M. Gueudin, H. Agut, and A. Gautheret-Dejean.** 2006. Identification of human herpesvirus 6 variants A and B by primer-specific real-time PCR may help to revisit their respective role in pathology. J. Clin. Virol. **35:**257–263.
6. **Caserta, M. T., C. B. Hall, K. Schnabel, K. McIntyre, C. Long, M. Costanza, S. Dewhurst, R. Insel, and L. G. Epstein.** 1994. Neuroinvasion and persistence of human herpesvirus 6 in children. J. Infect. Dis. **170:**1586–1589.
7. **Caserta, M. T., D. J. Mock, and S. Dewhurst.** 2001. Human herpesvirus 6. Clin. Infect. Dis. **33:**829–833.
8. **Chalfie, M., Y. Tu, G. Euskirchen, W. W. Ward, and D. C. Prasher.** 1994. Green fluorescent protein as a marker for gene expression. Science **263:**802–805.
9. **Clark, D. A., M. Ait-Khaled, A. C. Wheeler, I. M. Kidd, J. E. McLaughlin, M. A. Johnson, P. D. Griffiths, and V. C. Emery.** 1996. Quantitation of human herpesvirus 6 in immunocompetent persons and post-mortem tissues from AIDS patients by PCR. J. Gen. Virol. **77:**2271–2275.
10. **Clark, D. A., E. P. Nacheva, H. N. Leong, D. Brazma, Y. T. Li, E. H. F. Tsao, H. C. E. Buyck, C. E. Atkinson, H. M. Lawson, M. N. Potter, and P. D. Griffiths.** 2006. Transmission of integrated human herpesvirus 6 through stem cell transplantation: implications for laboratory diagnosis. J. Infect. Dis. **193:**912–916.
11. **Cuomo, L., A. Angeloni, C. Zompetta, M. Cirone, A. Calogero, L. Frati, G. Ragona, and A. Faggioni.** 1995. Human herpesvirus 6 variant A, but not variant B, infects EBV positive B lymphoid cells, activating the latent EBV genome through a BZLF-1 dependent mechanism. AIDS Res. Hum. Retrovir. **11:**1241–1245.
12. **Cuomo, L., P. Trivedi, M. R. Cardillo, F. M. Gagliardi, A. Vecchione, R. Caruso, A. Calogero, L. Frati, A. Faggioni, and G. Ragona.** 2001. Human herpesvirus 6 infection in neoplastic and normal brain tissue. J. Med. Virol. **63:**45–51.
13. **Daibata, M., T. Taguchi, Y. Nemoto, H. Taguchi, and I. Miyoshi.** 1999. Inheritance of chromosomally integrated human herpesvirus 6 DNA. Blood **94:**1545–1549.
14. **De Bolle, L., L. Naesens, and E. De Clercq.** 2005. Update on human herpesvirus 6 biology, clinical features, and therapy. Clin. Microbiol. Rev. **18:**217–245.
15. **Denes, E., L. Magy, K. Pradeau, S. Alain, P. Weinbreck, and S. Ranger-Rogez.** 2004. Successful treatment of human herpesvirus 6 encephalomyelitis in immunocompetent patient. Emerg. Infect. Dis. **10:**729–731.
16. **Dewhurst, S.** 2004. Human herpesvirus type 6 and human herpesvirus type 7 infections of the central nervous system. Herpes **11**(Suppl. 2)**:**105A–111A.
17. **Dominguez, G., T. R. Dambaugh, F. R. Stamey, S. Dewhurst, N. Inoue, and P. E. Pellett.** 1999. Human herpesvirus 6B genome sequence: coding content and comparison with human herpesvirus 6A. J. Virol. **73:**8040–8052.
18. **Flamand, L., I. Stefanescu, D. V. Ablashi, and J. Menezes.** 1993. Activation of the Epstein Barr virus replicative cycle by human herpesvirus 6. J. Virol. **67:**6768–6777.
19. **Gautheret-Dejean, A., C. Manichanh, F. Thien-Ah-Koon, A. M. Fillet, N. Mangeney, M. Vidaud, N. Dhedin, J. P. Vernant, and H. Agut.** 2002. Development of a real-time polymerase chain reaction assay for the diagnosis of human herpes-6 infection and application to bone marrow transplant patients. J. Virol. Methods **100:**27–35.
20. **Gompels, U. A., J. Nicholas, G. Lawrence, M. Jones, B. J. Thomson, M. E. Martin, S. Efstathiou, M. Craxton, and H. A. Macaulay.** 1995. The DNA sequence of human herpesvirus-6: structure, coding content, and genome evolution. Virology **209:**29–51.
21. **Gompels, U. A.** 2004. Roseolaviruses: human herpesviruses 6 and 7, p. 147–168. *In* A. J. Zuckerman, J. E. Banatvala, J. R. Pattison, P. D. Griffiths, and B. D. Schoub (ed.), Principles and practice of clinical virology, 5th ed. Wiley, London, United Kingdom.
22. **Hall, C. B., M. T. Casserta, K. C. Schnabel, C. Long, L. G. Epstein, R. A. Insel, and S. Dewhurst.** 1998. Persistence of human herpesvirus 6 according to site and variant: possible greater neurotropism of variant A. Clin. Infect. Dis. **26:**132–137.

23. **Hall, C. B., C. E. Long, K. C., Schnabel, M. T. Caserta, K. M. McIntyre, M. A. Costanzo, A. Knott, S. Dewhurst, R. A. Insel, and L. G. Epstein.** 1994. Human herpesvirus-6 infection in children: a prospective study of complications and reactivation. N. Engl. J. Med. **331:**432–438.
24. **Huang, L. M., C. Y. Lee, P. I. Lee, J. M. Chen, and P. J. Wang.** 1991. Meningitis caused by human herpesvirus-6. Arch. Dis. Child. **66:**1443–1444.
25. **Huang, L. M., C. Y. Lee, L. My, and P. I. Lee.** 1997. Primary infections of human herpesvirus-7 and herpesvirus-6: a comparative, longitudinal study up to 6 years of age. Acta Paediatr. **86:**604–608.
26. **Ihira, M., T. Yoshikawa, K. Suzuki, M. Ohashi, S. Suga, K. Horibe, N. Tanaka, H. Kimura, S. Kojima, K. Kato, T. Matsuyama, Y. Nishiyama, and Y. Asano.** 2002. Monitoring of active HHV-6 infection in bone marrow transplant recipients by real time PCR; comparison to detection of viral DNA in plasma by qualitative PCR. Microbiol. Immunol. **46:**701–705.
27. **Isaacson, E., C. A. Glaser, B. Forghani, Z. Amad, M. Wallace, R. W. Armstrong, M. M. Exner, and S. Schmid.** 2005. Evidence of human herpesvirus 6 infection in 4 immunocompetent patients with encephalitis. Clin. Infect. Dis. **40:**890–893.
28. **Isegawa, Y., T. Mukai, K. Nakano, M. Kagawa, J. Chen, Y. Mori, T. Sunagawa, K. Kawanashi, J. Sashihara, A. Hata, P. Zou, H. Kosuge, and K. Yamanishi.** 1999. Comparison of the complete DNA sequences of human herpesvirus 6 variants A and B. J. Virol. **73:**8053–8063.
29. **Ishiguro, N., S. Yamada, T. Takahashi, Y. Takahashi, T. Togashi, T. Okuno, and K. Yamanishi.** 1990. Meningo-encephalitis associated with HHV-6 related exanthem subitum. Acta Paediatr. Scand. **79:**987–989.
30. **Kearns, A. M., A. J. Turner, C. E. Taylor, P. W. George, R. Freeman, and A. R. Gennery.** 2001. LightCycler-based quantitative PCR for rapid detection of human herpesvirus 6 DNA in clinical material. J. Clin. Microbiol. **39:** 3020–3021.
31. **Krueger, G. R., B. Koch, N. Leyssens, Z. Berneman, J. Rojo, C. Horwitz, T. Sloots, M. Margalith, J. D. Conradis, S. Imai, I. Urasinsi, M. de Bruyere, V. Ferrer Argote, and J. Kreuger.** 1998. Comparison of seroprevalences of human herpesvirus-6 and -7 in healthy blood donors from nine countries. Vox Sang. **75:**193–197.
32. **Ljungman, P.** 2002. Betaherpes challenges in the transplant recipient. J. Infect. Dis. **186**(Suppl. 1)**:**S99–S109.
33. **Luppi, M., R. Marasca, P. Barozzi, S. Ferrari, L. Ceccherini-Nelli, G. Batoni, E. Merelli, and G. Torelli.** 1993. Three cases of human herpesvirus-6 latent infection: integration of viral genome in peripheral blood mononuclear cell DNA. J. Med. Virol. **40:**44–52.
34. **Lusso, P., and R. C. Gallo.** 1995. Human herpesvirus 6 in AIDS. Immunol. Today **16:**67–71.
35. **McCullers, J. A., F. D. Lakeman, and R. J. Whitley.** 1995. Human herpesvirus 6 is associated with focal encephalitis. Clin. Infect. Dis. **21:**571–576.
36. **Novoa, L. J., R. M. Nagra, T. Nakawatase, T. Edwards-Lee, W. W. Tourtellotte, and M. E. Cornford.** 1997. Fulminant demyelinating encephalomyelitis associated with productive HHV-6 infection in an immunocompetent adult. J. Med. Virol. **52:**301–308.
37. **Razonable, R. R., and C. V. Paya.** 2002. The impact of human herpesvirus-6 and -7 infection on the outcome of liver transplantation. Liver Transpl. **8:**651–658.
38. **Safronetz, D., A. Humar, and G. A. Tipples.** 2003. Differentiation and quantitation of human herpes viruses 6A, 6B and 7 by real-time PCR. J. Virol. Methods **112:**99–105.
39. **Saxinger, C., H. Polesky, N. Eby, S. Grufferman, R. Murphy, G. Tegtmeir, V. Parekh, S. Memon, and C. Huang.** 1988. Antibody reactivity with HBLV (HHV-6) in U.S. populations. J. Virol. Methods **21:**199–208.
40. **Singh, N., and D. L. Paterson.** 2000. Encephalitis caused by human herpesvirus-6 in transplant recipients: relevance of a novel neurotropic virus. Transplantation **69:**2474–2479.
41. **Takanashi, J., A. J. Barkovich, H. Tada, N. Takada, K. Fujii, and Y. Kohno.** 2006. Cortical liquefaction in severe human herpesvirus 6 encephalopathy. Neurology **66:**452–453.
42. **Tanaka-Taya, K., J. Sashihara, H. Kurahashi, K. Amo, H. Miyagawa, K. Kondo, S. Okada, and K. Yamanishi.** 2004. Human herpesvirus 6 (HHV-6) is transmitted from parent to child in an integrated form and characterization of cases with chromosomally integrated HHV-6 DNA. J. Med. Virol. **73:**465–473.
43. **Tokimasa, S., J. Hara, Y. Osugi, H. Ohta, Y. Matsuda, H. Fujisaki, A. Sawada, J. Y. Kim, J. Sashihara, K. Amou, H. Miyagawa, K. Tanaka-Taya, K. Yamanishi, and S. Okada.** 2002. Ganciclovir is effective for prophylaxis and treatment of human herpesvirus-6 in allogeneic stem cell transplantation. Bone Marrow Transplant. **29:**595–598.
44. **Van Den Bosch, G., G. Locatelli, L. Geerts, G. Faga, M. Ieven, H. Goossens, D. Bottiger, B. Oberg, P. Lusso, and Z. N. Berneman.** 2001. Development of reverse transcriptase PCR assays for detection of active human herpesvirus 6 infection. J. Clin. Microbiol. **39:**2308–2310.
45. **Yamanishi, K., T. Okuno, K. Shiraki, M. Takahashi, T. Kondo, Y. Asano, and T. Kurata.** 1988. Identification of human herpesvirus-6 as a casual agent of exanthem subitum. Lancet **i:**1065–1067.
46. **Yoshida, M., M. Yamada, T. Tsukazaki, S. Chatterjee, F. D. Lakeman, S. Nii, and R. J. Whitley.** 1998. Comparison of antiviral compounds against human herpesvirus 6 and 7. Antivir. Res. **40:**73–84.