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Survey for the Presence and Distribution of Human Herpesvirus 8 in Healthy Brain

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Brain tissues from both sides of the cerebellum and frontal, temporal, parietal, and occipital lobes were collected postmortem from 30 patients for human herpesvirus 8 (HHV-8) detection by PCR. Overall, 42 of 300 (14.0%) samples were positive, with similar rates for each position. Nineteen patients (63.3%) were positive and showed a significant increase in positivity with age ($P = 0.007$). The results indicate a neuroinvasive and neuropersistent potential of HHV-8.

Human herpesvirus 8 (HHV-8), also known as Kaposi's sarcoma (KS)-associated herpesvirus, is a new member of the *Rhadinovirus* genus in the *Gammaherpesvirinae* subfamily (5). HHV-8 is associated with all forms of KS in both immunocompromised and immunocompetent patients (2, 13). The virus has been found in primary effusion lymphoma (4, 11) and multicentric Castleman's disease (7). HHV-8 DNA can also be detected in noninvolved tissues from KS patients, including skin, peripheral blood mononuclear cells, lymphoid tissue, prostate, and semen (10, 14, 15). While the spectrum of in vivo tropism of HHV-8 has yet to be fully elucidated, reports of detection of HHV-8 in dorsal root ganglia of KS patients (6), cerebrospinal fluid from human immunodeficiency virus-positive patients (3), and brain biopsy specimens from patients with unexplained encephalitis (12) suggest a neuroinvasive potential of this novel virus. In this study, the prevalence and distribution of HHV-8 in brain were examined.

Study samples. Thirty consecutive unselected postmortem cases were included. For each patient, a fresh autopsy sample was collected from the cerebellum and frontal, temporal, parietal, and occipital lobes of both sides of the brain. Heart and kidney tissues of the same patient were also collected as controls. Hemorrhagic areas and areas with visible blood vessels were excluded. The study was approved by the local institutional ethics committee.

HHV-8 detection. DNA was extracted from a 2-mm^3 tissue block by the QIAamp Tissue Kit (Qiagen, Hilden, Germany), with the quality confirmed by a single-round PCR using primers PC03-PC07 targeting a 355-bp fragment of the β-globin gene as previously described (9). HHV-8 DNA was detected by a nested PCR (outer primers KS-1–KS-2, 5′-AGC CGA AAG GAT TCC ACC AT-3′ and 5′-TTC GTG TTG TCT ACG TCC AG-3′; inner primers H8NS1-H8NS2, 5′-ACG GAT TTG ACC CCG TGT TC-3′ and 5′-AAT GAC ACA TTG GTG GTA TA-3′) targeting a 233-bp and a 160-bp fragment, respectively, of open reading frame (ORF) 26 (5, 10). Five microliters of extracted DNA was amplified in a 50-μl reaction mixture containing PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, and 1.5 mM $MgCl_2$), 200 μM (each) deoxynucleoside triphosphates, 1 U of *Taq* polymerase (Pharmacia Biotech, Uppsala, Sweden), and 0.25 μM (each) outer primers. The cycling conditions were initial denaturation (94°C, 4 min); 30 cycles of denaturation (94°C, 1 min), annealing (55°C, 1 min), and extension (72°C, 1 min); and a final extension (72°C, 8 min). Two microliters of the first-round amplicons was amplified in a second-round PCR with the same conditions using inner primers, 55°C for annealing, and omission of initial denaturation. PCR amplicons were electrophoresed and visualized by ethidium bromide staining. All reactions were carried out under stringent conditions to avoid cross-contamination (8). A negative control was included following each fifth sample.

Purified DNA from each of the eight human herpesviruses was used to assess the specificity of HHV-8 PCR, and no cross-amplification from other human herpesviruses was observed (data not shown). In addition, the specificity of amplicons was confirmed by another nested PCR (outer primers, 5′-AGG CAA CGT CAG ATG TGA C-3′ and 5′-GAA ATT ACC CAC GAG ATC GA-3′; inner primers, 5′-CAT GGG AGT ACA TTG TCA GGA CCT C-3′ and 5′-GGA ATT ATC TCG CAG GTT GCC-3′) targeting a 328-bp and a 213-bp fragment, respectively, of ORF 25 (1).

Statistical analysis. The chi-square test or Fisher's exact test was used to analyze categorical variables. The independent-sample *t* test was used for numerical variables. Two-tailed *P* values of <0.05 were regarded as significant.

The 30 studied patients (29 Chinese and 1 Portuguese) were aged 20 to 95 years (mean, 61.4; standard deviation, 20.2) with a male/female ratio of 2:1. Three patients died of intracranial hemorrhage, with one due to trauma and two from rupture of intracranial arterial aneurysms. The deaths of remaining patients were not related to the central nervous system. None of the patients had clinical or pathological findings suggestive of current viral infection or skin lesions compatible with KS.

All extracted DNAs were positive by the β-globin PCR. All PCR controls, including heart and kidney tissues, were negative in the HHV-8 PCR. Samples positive by the first set of HHV-8 PCR (ORF 26) were all confirmed by the second set of HHV-8 PCR (ORF 25).

Overall, 300 brain tissue samples were examined with 42 (14.0%) being positive for HHV-8 DNA. The positive rates for each anatomical position were similar (Table 1). Nineteen patients (63.3%) had HHV-8 DNA detected, of which 10 (52.6%) harbored viral DNA at more than one anatomical position, including five patients with two positive samples, one patient with three positive samples, and three patients with four positive samples. A 78-year-old male patient who died of

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TABLE 1. Distribution of HHV-8 DNA in brain

Anatomical position	No. (%) of specimens positive for HHV-8 DNA[a]		
	Left side	Right side	Total
Cerebellum	3 (10.0)	6 (20.0)	9 (15.0)
Frontal lobe	5 (16.7)	5 (16.7)	10 (16.7)
Temporal lobe	5 (16.7)	3 (10.0)	8 (13.3)
Parietal lobe	3 (10.0)	3 (10.0)	6 (10.0)
Occipital lobe	4 (13.3)	5 (16.7)	9 (15.0)
Total	20 (13.3)	22 (14.7)	42 (14.0)

[a] One specimen was collected from each position of the 30 studied patients.

hemothorax due to traumatic injury harbored viral DNA at eight anatomical sites. The viral DNA-positive and -negative groups showed no significant difference in sex distribution (male/female ratio, 11:8 versus 9:2; $P = 0.246$ by Fisher's exact test). Patients positive for HHV-8 were significantly older than the negative group (mean age, 68.2 versus 49.6 years; $P = 0.012$; 95% confidence interval, 4.32 to 32.73 by t test). A significant trend of increase in positive rate with age was also observed (age < 30 years, 0%; 30 to 49 years, 42.9%; 50 to 69 years, 63.6%; >69 years, 90.0%; χ^2 for linear trend, 7.336; $P = 0.007$).

The interpretation of detection of a lymphotropic virus, like HHV-8, in any tissue where the microvasculature cannot be avoided may be difficult. In this study, it is unlikely that the positive PCR results were due to HHV-8 carrying lymphocytes within the microvasculature of brain tissues, since all control tissues were negative by the HHV-8 PCR. Our results show that HHV-8 DNA can be detected in brain tissues of a majority (63.3%) of the adult population in Hong Kong. In most individuals, HHV-8 can be found in more than one part of the brain, but there seems to be no predilection for any particular position within the brain. Whether the observed trend of increase in positivity with age is related to an increase in seroprevalence remains to be established. Although data on seroprevalence of HHV-8 in Hong Kong are not yet available, the results of this study suggest that HHV-8 is highly prevalent among our Chinese population. Our findings indicate that HHV-8 carries a neuroinvasive and neuropersistent potential, and the central nervous system may be another site for viral latency. While the potential role of HHV-8 in neuropathology, particularly during its reactivation from brain tissue, cannot be neglected, the fact that HHV-8 sequences are present in a majority of the adult population should be considered in the interpretation of its pathologic significance.

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