

Original
articleHerpesvirus infection of eye and brain in HIV
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Objectives: To compare histological with genome detection methods for diagnosis of herpesvirus infection in eye and brain of HIV infected patients undergoing necropsy and to correlate these findings with both antemortem clinical findings and postmortem evidence of extraocular herpesvirus infection, especially in the CNS.

Methods: A prospective study of 31 consecutive HIV infected patients undergoing necropsy. In life 11 patients had been assessed by an ophthalmologist because of ocular symptoms. Ocular and brain samples were examined for herpesviruses by conventional histological methods and by nested polymerase chain reaction (nPCR) for all eight human herpesviruses; evidence of extraneural herpesvirus infection was sought by histological methods.

Results: Although only 12 out of 31 patients (39%) had antemortem clinical evidence of ocular or CNS herpesvirus associated disease, herpesviruses were detected by nPCR in eye and brain from 26 (84%) patients; six patients had more than one herpesvirus infection. There was concordance between ocular and CNS findings in 15 of 19 patients (79%) with CMV infection. 17 of 31 patients (55%) had extraocular or CNS CMV infection at necropsy. Genome detection using nPCR was superior to histological methods for diagnosis of ocular and CNS herpesvirus infection.

Conclusion: Herpesvirus infection of eye and brain was a frequent finding at necropsy in this group of HIV infected patients; almost a fifth were co-infected by more than one herpesvirus. This was more than twice the incidence predicted from clinical evidence before death. Genome detection using nPCR was superior to histological methods for diagnosis of ocular and CNS herpesvirus infection.

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Keywords: AIDS; necropsy; herpesviruses; DNA detection; CMV retinitis

Introduction

Herpesviruses are well described causes of ocular and central nervous system (CNS) disease in immunocompromised HIV infected patients.^{1,2} While cytomegalovirus is the most common cause of retinitis,³ necrotising herpetic retinopathy due to varicella zoster virus or herpes simplex virus has also been described.^{1,4} Cytomegalovirus and herpes simplex virus may also cause encephalitis,⁵ and varicella zoster virus may cause meningitis and/or encephalitis.⁶ Of the other herpesviruses, Epstein–Barr virus is strongly associated with CNS non-Hodgkin's lymphoma.⁷ Human herpesvirus-6 has been identified in the retina of AIDS patients without retinitis,⁸ and human herpesvirus-8 is strongly associated with Kaposi's sarcoma⁹; it is also associated with encephalitis.¹⁰ The association between human herpesvirus-8 and CNS lymphoma is uncertain.¹¹

In HIV infected patients, detection of herpesviruses from ocular samples (vitreous and aqueous fluid) and from cerebrospinal fluid and brain tissue by DNA amplification using nested polymerase chain reaction (nPCR) has been shown to be a rapid, highly sensitive, and specific technique.^{2,4–7,9–12}

The neurological/neuropathological complications of HIV infection are well described. In contrast, while there are many clinical descrip-

tions of HIV associated ocular disease there are few reports of unselected case series with clinicopathological correlation.¹³ Further, the data on simultaneous pathological changes in brain and eye in patients with AIDS are largely made up of single cases.^{14–16} In one necropsy based consecutive case series of 43 AIDS patients from Copenhagen only 14% had concomitant pathological lesions in brain and eye, due to CMV infection or B cell lymphoma.¹⁷ However, the study did not use genome detection methods for virus detection.

In this study the aim was to determine at necropsy the prevalence of ocular and CNS herpesvirus infections in patients with AIDS, by means of histological and genome detection methods, and to correlate these findings with antemortem clinical features and the presence of postmortem extraocular or CNS herpesvirus infection. In order to reduce a potential selection bias, a consecutive series of HIV infected patients undergoing necropsy was studied, irrespective of whether or not they had ocular disease diagnosed in life.

Methods

PATIENTS

We prospectively studied 31 consecutive HIV infected patients who underwent necropsy at University College London Hospitals during a 16 month period from December 1993 to

March 1995. The cranial neuroradiology/neuropathology of some of these patients has been described elsewhere.^{18, 19} In life all had been under the care of an HIV specialist (RFM) and had well documented clinical disease. Clinical assessment had included ophthalmological examination, performed between 2 and 65 days (median 14 days before death). In life 11 patients who had ocular symptoms had also been examined by indirect ophthalmoscopy after full dilatation of pupils by a medical ophthalmologist (PF) experienced in the diagnosis of retinal disease in HIV infected patients. Of the 31 patients, 29 were men; 28 were white, of whom 26 were homosexual and two were injecting drug users, one was of African origin and was heterosexual. The two women were white and heterosexual. Their ages ranged from 27 to 53 (median 36) years and CD4 lymphocyte counts ranged from 0 to 380 cells $\times 10^6/l$ (median 10 cells $\times 10^6/l$) (normal range 350–2200 cells $\times 10^6/l$). In all but one patient the CD4 lymphocyte count was ≤ 110 cells $\times 10^6/l$. Written consent for necropsy was obtained for all patients, with permission to remove brain and one or both eyes.

PATHOLOGY

All necropsies were performed by one pathologist (SBL) using a standardised protocol.^{19, 20} The interval between death and necropsy was 1 (0–3) day (median (range)). Globes were removed intact and aqueous and vitreous fluids were aspirated for molecular analysis (see below). Brains were removed intact and in 12 patients a 0.5 cm³ section of frontal lobe was removed, placed in a cryotube, and snap frozen in liquid nitrogen and stored at -70°C for analysis by nPCR (see below). In the remaining 19 patients CSF was obtained.

Globes and brain were fixed in formal saline for 2–4 weeks. Multiple tissue samples were taken from the fixed brain, using a previously described protocol,¹⁹ and processed for light microscopy as previously described.¹⁹ Globes were sectioned horizontally and embedded in paraffin. Multiple sections were cut and stained with haematoxylin and eosin, Luxol fast blue, and cresyl violet. In each case the retina, choroid, iris, and optic nerve were examined. Selected sections were also stained with Ziehl–Neelsen, periodic acid Schiff, Van Gieson, and Grocott's methenamine silver stains.

Ocular, brain, and extraneural tissues were specifically examined for histological evidence of CMV infection by presence of typical intranuclear and intracytoplasmic inclusions on haematoxylin and eosin stains associated with focal inflammation and necrosis. In some cases immunocytochemistry for detection of CMV antigen was also performed.

MOLECULAR STUDIES

DNA extraction

Aqueous and vitreous fluid were centrifuged (15 000 *g* for 5 minutes) to separate cells from supernatant. Cell free supernatant was boiled for 10 minutes, cooled on ice, and stored at -20°C until used. After thawing frontal lobe

tissue was ground under liquid nitrogen with a pestle and mortar and the homogenate resuspended in a solution containing 0.10 mg/ml proteinase K, 0.01 M TRIS, pH = 8.0, 0.1M sodium chloride, 0.01 M EDTA, and 0.5% (w/v) sodium dodecyl sulphate (SDS). Protein digestion was achieved by incubation of the mixture at 60°C for 2 hours (to inactivate HIV) then at 37°C for 12 hours. Lysis was stopped by boiling for 10 minutes to inactivate proteinase K and placed on ice, then spun at 3000 *g* for 1 minute. Supernatant was then diluted 1:10 in distilled water. This dilution step avoids inhibition of PCR by SDS (data not shown).

Nested polymerase chain reaction

Input volumes for the first round of PCR were 10 μl of CSF, diluted brain extract or aqueous fluid, or 1 μl of vitreous fluid. The nPCR method used to detect herpes simplex type 1 (HSV-1) *gD*, herpes simplex type 2 (HSV-2) *gG*, varicella zoster virus (VZV) *gene 29*, Epstein–Barr virus (EBV) internal repeats, cytomegalovirus (CMV) *gB*, human herpesvirus-6 (HHV-6) 13R, herpesvirus-7 (HHV-7) U42, and human herpesvirus-8 (HHV-8) minor capsid were as previously described.^{9, 12, 21–23}

STATISTICAL ANALYSES

Non-parametric data were compared using Wilcoxon's rank sum test. A *p* value of <0.05 was considered statistically significant.

Results

Overall, herpesvirus was detected in the eye and/or brain of 84% (26/31) of the patients studied.

CYTOMEGALOVIRUS

Clinically, 8/31 patients had CMV retinitis, necropsy pathology revealed a further two cases (10/31) and nPCR analysis another seven ophthalmic CMV infections (total 17/31 (55%), fig 1A). Necropsy pathology showed active CMV infection with intranuclear and intracytoplasmic inclusions, variable inflammation and old necrosis. Additional non-herpesvirus pathology was detected at necropsy in two patients with CMV retinitis and in 10 without retinitis (table 1). CMV DNA was detected in both aqueous and vitreous fluid in all 10 with pathological evidence of CMV retinitis and also from seven other patients without clinical or pathological evidence of CMV ocular disease. Thus, we found the sensitivity of CMV DNA detection in ocular fluid for diagnosis of CMV retinitis was 100% and the specificity was 66%. The relation between clinical, histological, and genome detection methods for diagnosis of ocular CMV infection is shown in figure 1A.

Cytomegalovirus encephalitis was diagnosed in 10 patients at necropsy. Findings were intranuclear and intracytoplasmic inclusions with associated focal inflammation and necrosis, microglial nodules, and necrotising ventricular ependymitis. In six of these patients a diagnosis had been made in life, on the basis of features of a subacute onset of cognitive deficit¹⁹ together with antemortem detection of CMV

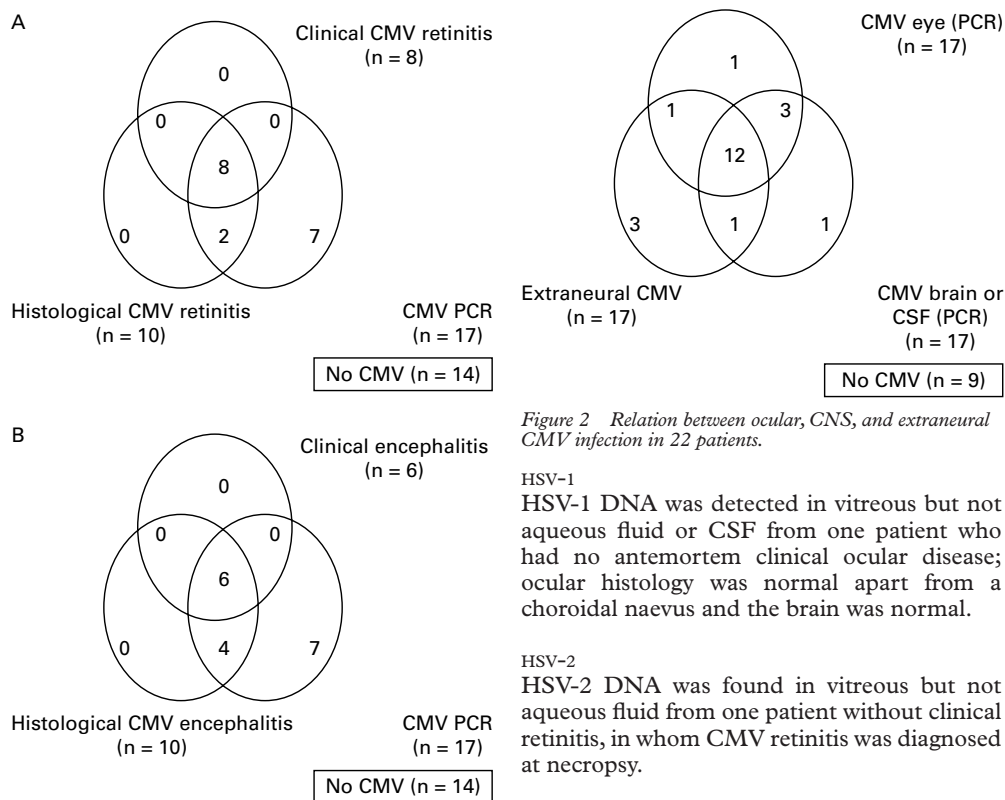


Figure 2 Relation between ocular, CNS, and extraneural CMV infection in 22 patients.

Figure 1 (A) Diagnosis of CMV infection of eye in 17 patients with CMV DNA detected in ocular samples. (B) Diagnosis of CMV infection of brain in 17 patients with detectable CMV DNA in CSF or brain.

Table 1 Non-herpesvirus related ocular pathology in 31 patients with and without histologically diagnosed CMV retinitis

Pathological findings	CMV retinitis	
	Present (n=10)	Not detected (n=21)
Non-specific choroiditis	0	5
Anterior uveitis	0	2
Choroidal <i>Cryptococcus neoformans</i>	1	1
Choroidal <i>Mycobacterium tuberculosis</i>	1	0
<i>Toxoplasma gondii</i> choroiditis	0	1
Choroidal naevus	0	1
Normal	8	11

DNA in CSF by nPCR. CMV DNA was detected in brain or postmortem CSF of these 10 patients and also in seven other patients without histological evidence of CNS CMV infection (fig 1B).

In life none of the 31 patients had evidence of extraneural CMV organ disease; necropsy showed 17 patients had disseminated CMV disease; seven of these had isolated CMV adenitis. The relation between detection of CMV by nPCR in ocular and CNS samples and histologically at extraneural sites is shown in figure 2. There was no difference in the interval between the last clinical/ophthalmological assessment and death in those with widespread herpesvirus infection = 3–65 (14) days (range (median)) and those without = 2–59 (13) days. Wilcoxon's rank sum test; $p = NS$.

HSV-1

HSV-1 DNA was detected in vitreous but not aqueous fluid or CSF from one patient who had no antemortem clinical ocular disease; ocular histology was normal apart from a choroidal naevus and the brain was normal.

HSV-2

HSV-2 DNA was found in vitreous but not aqueous fluid from one patient without clinical retinitis, in whom CMV retinitis was diagnosed at necropsy.

VZV

VZV DNA was detected in CSF and aqueous fluid of one patient and in three other patients VZV DNA was detected in CSF; in these three CMV was also detected in CSF. None of these four had antemortem or necropsy evidence of ocular, brain, or spinal cord VZV disease.

EBV

EBV DNA was detected in CSF or brain extract of six patients; in one EBV DNA was also detected in aqueous but not vitreous fluid. At necropsy three of these six patients had CNS but not ocular lymphoma (two of these had been diagnosed in life). The three others did not have lymphoma, their diagnoses were focal pontine leucoencephalopathy, *Toxoplasma gondii* encephalitis without abscess formation, and HIV leucoencephalopathy with multinucleate giant cell encephalitis.

HHV-8

HHV-8 DNA was detected in three patients without ocular disease at necropsy. It was present in aqueous fluid of one patient, in another in brain extract, and both aqueous and vitreous fluid, and in brain extract, together with CMV DNA in a third patient who had CMV encephalitis. All three patients had disseminated cutaneous and visceral Kaposi's sarcoma at necropsy. None had evidence of ocular, CNS, or extraneural lymphoma.

HHV-6 AND 7

No HHV-6 or HHV-7 DNA was detected in any ocular or CNS samples examined.

Discussion

This study sought to compare clinical, histological, and genome detection methods for

identification of herpesvirus infection in eyes and brain of a consecutive series of HIV infected patients who underwent necropsy and to correlate these findings with antemortem features and with evidence of postmortem extraocular and extraneural herpesvirus infection.

There were three striking findings in the current study. First was the high frequency of detection of herpesviruses (84%) in eye and brain of this group of profoundly immunosuppressed HIV infected patients, CMV in 61%, HSV-1/2 in 6%, VZV in 13%, EBV in 19%, and HHV-8 in 10%. Yet, in contrast, only 10 patients (32%) had herpesvirus associated disease diagnosed antemortem. In almost a fifth of our patients (19%) more than one herpesvirus was identified in a single patient. In contrast, Pillay *et al* performed a necropsy based clinicopathological study of 47 consecutive patients and searched for opportunistic viral infections in brain and extraneural tissues (but not eye) by histological methods including immunocytochemistry for CMV; in some patients conventional cell culture was also carried out.²⁴ Overall, 72% of patients had an opportunistic viral infection: CMV in 31 patients (66%) and HSV-1 in five (11%) were the most frequently identified pathogens. However, CMV was identified in brain of only eight (17%) and at extraneural sites, including lung in 19 patients and adrenal in 17 patients. Of those with CMV infection the diagnosis had been made antemortem in only 17/31 (54%).²⁴

The second striking finding was the high rate of concordance between ocular and CNS CMV disease which occurred in 15/19 (79%); only two patients each had either ocular or CNS disease alone. This finding contrasts with the results of a study by Jensen and Klinken where, of 43 consecutive patients with AIDS undergoing necropsy using conventional histological methods to diagnose herpesvirus infection, nine patients were found to have CMV retinitis and eight had CMV encephalitis. Only four (31%) had both diagnoses.¹⁷ In this study the presence of extraneural CMV disease was not recorded.

Previous studies have described finding CMV retinitis at necropsy in patients without antemortem clinically diagnosed disease, so in this regard our data are not unique.^{13 17 25} In a study from Stockholm,¹³ 17 of 48 consecutive HIV infected patients (36%) had an ocular abnormality at necropsy. In life 29 had undergone ophthalmological examination and 16 were thought clinically to have CMV retinitis. Necropsy identified CMV retinitis in 14 of the 16 and in one other patient undiagnosed in life. In the other 2/16 the necropsy diagnosis was *Toxoplasma gondii* retinochoroiditis, which in life had been mistakenly been diagnosed as CMV retinitis. Morinelli *et al* used conventional histology to examine at necropsy the eyes of 235 consecutive HIV infected patients.²⁵ Infectious choroiditis was identified in 18 patients (including *Cryptococcus neoformans* in seven and *Pneumocystis carinii* in four). Five patients also had CMV retinitis; the diagnosis was made antemortem in only three patients.

In one other patient CMV retinitis had been misdiagnosed in life and necropsy revealed only *Mycobacterium avium-intracellulare* choroiditis.²⁵

Thirdly, we found that genome detection using nPCR was superior to histological methods for identifying herpesviruses. As herpesviruses are strongly cell associated and establish latency it is clearly important to discriminate between infection and disease. With the methodology used in this study there is the potential for false positive results owing to contamination of samples by cells latently infected with herpesviruses—for example, peripheral blood mononuclear cells. We have previously demonstrated that the methodology used in this study performing nPCR on cell free supernatant of CSF obtained by centrifugation for detection of herpesviruses avoids the potential for cellular contamination.^{7 21 26} This is particularly important in the context of EBV infection as up to 61% of HIV infected patients with low CD4 lymphocyte counts have peripheral blood mononuclear cells that contain detectable EBV DNA.⁹

We found complete concordance for detection of CMV from both aqueous and vitreous fluid which suggests that either sample may be used for sampling. In this study nPCR and ocular samples had a high sensitivity for diagnosis of CMV retinitis but only a moderate specificity, as nPCR for CMV was positive in ocular samples from patients without CMV retinitis. This suggests that caution is necessary in interpreting results of CMV genome detection methods if they are used for diagnostic purposes as some patients with retinitis due to causes other than CMV—for example, *T. gondii*, syphilis, or necrotising herpetic retinopathy (due to HSV or VZV) may have misleadingly positive CMV nPCR results.

In three patients with CNS but not ocular lymphoma, EBV DNA was detected in CSF or brain extract; in one of these patients EBV DNA was also detected in an ocular sample. EBV DNA was also detected in CSF or brain extract from three other patients with alternative diagnoses and no lymphoma. Antemortem EBV DNA detection by nPCR in CSF of HIV infected patients is strongly associated with both primary and metastatic CNS lymphoma.⁷ Thus, the findings of the current study might imply a reduced specificity for EBV genome detection; however, antemortem EBV DNA in CSF from patients without lymphoma is predictive of its subsequent development,⁷ so it is possible that these three patients were at particularly high risk of developing lymphoma.

We identified VZV DNA in CSF of four patients (one also in aqueous fluid) without clinical or pathological evidence of disease. In one study²⁶ VZV DNA was identified in CSF of six (5%) of 120 consecutive HIV infected patients: all six had neurological symptoms and recent or concurrent cutaneous zoster.²⁶ This does not provide an explanation for our current observations as only one of the four patients had recent (<12 months) cutaneous zoster. In contrast, VZV DNA was detected in CSF of 13 (2.5%) of 514 consecutive HIV infected

patients undergoing diagnostic lumbar puncture. Whereas in four patients VZV DNA detection was associated with encephalitis or meningoencephalomyelitis, in the remaining nine neurological disease was caused by non-herpesvirus associated pathology.²⁷ The presence of VZV DNA in these cases was ascribed to subclinical reactivation of VZV infection.

In three of our patients HHV-8 DNA was detected in ocular fluid and/or brain, all three had disseminated Kaposi's sarcoma and none had CNS or extraneural lymphoma. Interpretation of this finding is not easy as the role of HHV-8 in the CNS of immunosuppressed patients remains unclear. In one prospective study of 115 HIV infected patients undergoing diagnostic lumbar puncture for investigation of acute neurological episodes HHV-8 DNA was detected in cell free CSF of two patients.⁷ Neither patient had neurological disease, one had systemic but not CNS lymphoma and no Kaposi's sarcoma, the other had disseminated Kaposi's sarcoma but no lymphoma. Corboy *et al* studied 36 patients immunosuppressed by HIV or malignancy who had CNS lymphoma diagnosed either by brain biopsy or at necropsy.¹¹ Twenty nine patients had primary CNS lymphoma (27 B cell and two T cell) and seven had metastatic CNS lymphoma (five B cell, one T cell, and one K 1 cell). HHV-8 DNA was identified in 15/27 with B cell primary CNS lymphoma, one of five with metastatic B cell lymphoma, and in none of the four with other lymphomas.¹¹ In contrast, Said *et al* reported three patients (two were HIV infected) with acute encephalitis; none had Kaposi's sarcoma or lymphoma. Using nPCR and Southern blotting HHV-8 DNA was detected in brain biopsy tissue from all three patients.¹⁰

In conclusion, evidence of herpesvirus infection of eye and brain was a frequent finding at necropsy in this group of HIV infected patients and was more than twice as common as antemortem clinical features suggested. Almost a fifth were co-infected by more than one herpesvirus. Genome detection using nPCR was superior to histological methods for identification of herpesvirus infection and showed that there was a high level of concordance between CMV infection of eye and brain.

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Contributors: All authors contributed to the design of the study, to data collection and analysis, and to writing drafts of the manuscript. MRH and CJP did the PCR analyses, SBL carried out the necropsies, examined the brains and together with IP examined the histopathology of eyes. RFM collated clinical, molecular, and pathological data and wrote the first and final drafts of the manuscript. The original research proposal was put forward by SBL and RFM.

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