Human Herpesvirus-6 Detection in Cerebrospinal Fluid on the BioFire FilmArray Meningitis/Encephalitis Panel in a High Human Immunodeficiency Virus-Prevalence African Setting

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The prevalence and clinical relevance of human herpesvirus-6 (HHV-6) detection in cerebrospinal fluid (CSF) using multiplex polymerase chain reaction (PCR) testing in patients with suspected meningocencephalitis in high human immunodeficiency virus-prevalence African settings are not known. We describe the clinical and laboratory characteristics of 13 patients with HHV-6 CSF PCR positivity in Botswana.

Keywords. cerebrospinal fluid; encephalitis; HIV; human herpesvirus-6; meningitis.

Human herpesvirus-6 (HHV-6) is a common cause of infantile fever and roseola and has been implicated as a central nervous system (CNS) pathogen causing meningitis or meningocencephalitis, predominantly in immunocompromised hosts [1]. Because multiplexed cerebrospinal fluid (CSF) polymerase chain reaction (PCR) testing is becoming more widely used in the diagnosis of suspected meningocencephalitis [2], HHV-6 PCR positivity in the CSF is increasingly reported [2, 3]. However, its clinical significance remains unclear [4, 5]. Prior reports from United States and Germany have shown that HHV-6 comprises between 10.7% and 31.3% of all positive CSF BioFire FilmArray Meningitis/Encephalitis (FilmArray-ME) panel results among patients with suspected meningocencephalitis [2, 3]. The majority of these HHV-6-positive results were in children [2], and overall HHV-6 was the second most frequently encountered viral pathogen after enterovirus [2, 3]. To date, there are very limited data on the prevalence and clinical correlates of CSF HHV-6 detection in sub-Saharan Africa [6, 7] where the etiology of meningocencephalitis differs markedly from Europe and North America [8], and a large proportion of individuals presenting with meningocencephalitis have immune suppression due to human immunodeficiency virus (HIV), and culture-negative lymphocytic meningitis is common and associated with high mortality [9].

As part of a the prospectively Botswana National Meningitis Survey [9], we implemented multiplex PCR CSF testing using the FilmArray-ME Panel at the main referral hospital in Gaborone. Human herpesvirus-6 was the second most frequently detected virus after cytomegalovirus. We describe the clinical and laboratory characteristics of the 13 patients with HHV-6 detected on CSF analysis.

METHODS

Cerebrospinal fluid samples from 690 sequential patients presenting to Princess Marina Hospital, Gaborone, between April 2017 and November 2018, were analyzed using the BioFire FilmArray Meningitis/Encephalitis multiplex PCR panel (bioMérieux, Grenoble, Rhone-Alpes, France) (Appendix 1). Clinical and laboratory data and in-hospital outcomes were captured using REDCap. A consensus diagnosis was attributed to each patient, and the relevance of HHV-6 detection in the CSF on FilmArray-ME and its role as a CNS pathogen was classified as "likely", "possible", or "unlikely" (adapted from previously described criteria, outlined in Table 1 [4], following detailed records review by 2 independent clinicians [J.M. and C.G.W.], with arbitration by a third [J.N.J.]). Ethical approvals for the study were provided by the Botswana Health Research and Development Administration by a third [J.M. and C.G.W.].

RESULTS

Of the 690 patients with CSF multiplex PCR analysis, at least 1 organism was identified on PCR in 124 (18%). Human herpesvirus-6 was detected in the CSF of 13 patients, comprising 11% of all positive results (Table 1). Six of the HHV-6-positive individuals were infants under 1 year of age (of a total of 225 tested with FilmArray-ME), 1 was a 12-year-old child (of 45 children...
| Patient | Age and Sex | Presenting Complaint / Clinical Syndrome | HIV Status | CD4 Count | CSF Cell Count (Leucocytes/mm³) and Differential (%) | Antiretroviral Therapy | CSF Protein (mg/L) | CSF Glucose (mmol/L) | Hemoglobin (g/dL) | Peripheral WCC (10⁹/L) | Outcome on Admission | Additional Clinically Relevant Microbiological Results | Final Diagnosis | HHV-6 Meningoencephalitis (Unlikely/Possible/Likely) | Outcome at Discharge |
|---------|-------------|-----------------------------------------|------------|-----------|--------------------------------------------------|-----------------------|--------------------|-------------------|-----------------|--------------------|---------------------|-----------------------------------------------|-----------------|-------------------------------------------|-------------------|
| 1       | 2 months old | Vomiting                                | Unexposed  | 2         | n/a                                              | n/a                   | 0.29               | 2.53              | 10              | 13.74              | No                  | Nil                                           | Nil             | Gastroenteritis possibly primary HHV-6 infection | Unlikely          | Alive                                   |
| 2       | 2 months old | Tachypnoea                              | Exposed    | 2         | n/a                                              | n/a                   | 0.22               | 3.44              | 7.0             | 10.70              | Yes                 | Nil                                           | Nil             | Pneumonia Possible primary HHV-6 infection   | Unlikely          | Alive                                   |
| 3       | 5 months old | Diarrhea and vomiting                   | Unexposed  | 2         | n/a                                              | n/a                   | 0.31               | 3.90              | 9.8             | 5.50               | Yes                 | Nil                                           | Nil             | Unidentified Gram-negative organism in CSF | Unlikely          | Alive                                   |
| 4       | 7 months old | Fever, seizures, lethargy, and diarrhea | Unexposed  | 2         | n/a                                              | n/a                   | 0.23               | 4.14              | 11.3            | 4.07               | No                  | Nil                                           | Nil             | Pneumonia complicated by febrile seizure | Unlikely          | Alive                                   |
| 5       | 7 months old | Fever, seizures, and URT symptoms       | Negative   | 2         | n/a                                              | n/a                   | 0.16               | 2.53              | 5.6             | 13.9               | No                  | Nil                                           | Nil             | Vertebral respiratory tract infection complicated by febrile seizure | Unlikely          | Alive                                   |
| 6       | 1 months old | Fever, rash, and cough                  | Negative   | 3         | n/a                                              | n/a                   | 0.19               | 4.27              | 11.4            | 5.09               | Yes                 | Nil                                           | Nil             | Possible primary HHV-6 infection            | Possible          | Alive                                   |
| 7       | 12 years old | Headache, neck stiffness, seizures, and left-sided weakness | Unknown   | 10        | Differential not performed                       | n/a                   | 0.51               | 2.1               | 12.3            | 6.93               | No                  | Nil                                           | Nil             | Coccaceae-negative staphylococcus cultured in CSF | Hydrocephalus       | Unlikely                                |
| 8       | 3 years old  | Altered mental status                   | Positive   | 4/0       | On ARVs                                          | 0.46                 | 3.29               | 9.7              | 3.07              | No                  | Nil                                           | Nil             | Psychiatric disorder                      | Unlikely          | Alive                                   |
| 9       | 35 years old | Cough, weight loss, and night sweats    | Positive   | Unknown   | 3                                                | 2.05                 | 5.95               | 8.3              | 22.42             | Yes                 | Nil                                           | Nil             | Pneumonia                                 | Unlikely          | Alive                                   |
| 10      | 40 years old | Left-sided weakness and slurred speech  | Positive   | 111       | 99% lymphocytes (1% polymorphs)                  | 0.81                 | 3.18               | 11               | 3.37              | No                  | Aciclovir VZV VZV meningitis + Mycobacteria | Unlikely          | Died during admission                       |                  |
| 11      | 49 years old | Headache, vomiting, weakness            | Positive   | Unknown   | 946 (89% lymphocytes 1% polymorphs)              | On ARVs              | Not performed      | Not performed     | 9.7              | 1.44               | Not                | Nil                                           | Positive CrAg in CSF | Cryptococcal meningitis | Unlikely          | Alive                                   |
Table 1. Continued

| Patient | Age and Sex | Presenting Complaint/Clinical Syndrome | HIV Status | CD4 Count | CSF Cell Count (Leucocytes/mm³) and Differentialb | Antiretroviral Therapy | CSF Protein (mg/L)c | CSF Glucose (mmol/L)d | Hemoglobin (g/dL)e | Peripheral WCC (10⁹/L)f | Objective ever on Admission | Antiviral Treatment | Additional Clinically Relevant Microbiological Results | Final Diagnosis | HHV-6 Meningoencephalitis | Outcome at Discharge |
|---------|-------------|----------------------------------------|------------|-----------|-----------------------------------------------|-----------------------|-------------------|------------------|----------------|------------------|----------------------|-----------------|----------------------------|------------------|----------------------|-------------------|
| 12      | 27 years male | Diabetes, altered mental status, and neck stiffness | Presentive | 1.07 | 250 | 88% lymphocytes/ 2% polymorph | Defaulted | 0.79 | 1.95 | 8.8 | 6.49 | No | Aciclovir | Nil | Possible tuberculous meningitis | Possible | Alive |
| 13      | 40 years male | Cough, headache, visual hallucination, and altered mental status | Presentive | 9 | 3 | Previously defaulted—likely HHV-6 | 1.15 | Not performed | Yes | Valganciclovir | Nil | Possible tuberculous meningitis | Possible | Alive |

Presented with 7-day history of headache, vomiting, and neck stiffness. On examination they were photophobic with oral thrush with signs of malnutrition. Opening pressure on lumbar puncture was 48 cm. CSF with a positive CrAg on CSF analysis. The patient was treated for cryptococcal meningitis and also treated empirically for TB. 

Presented with 1-month history of diarrhea, altered mental status, and neck stiffness. There was a history of advanced HIV disease having previously default treatment and a recent diagnosis of pulmonary TB 3 months before presentation but defaulted after approximately 4 weeks therapy. Treated for tuberculous meningitis and received ceftazidime for 9 days for possible bacterial meningitis. A doxycycline started after positive HHV-6 result (valganciclovir unavailable).

Presented with 2-week history of headache, hallucination, fever, and cough. No features of meningitis on examination. Previously defaulted on ART treatment. Started on ART and restarted on ART hands before admission when presented with symptoms suggestive of TB meningitis. Presente this admission with similar symptoms and ARVs held due to suspected IPS but were re-started on discharge. Treated with valganciclovir for possible HHV-6 meningoencephalitis.

Abbreviations: ART, antiretroviral drugs; ATT, antituberculosis therapy; CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; CT, computed tomographic; HIV, human immunodeficiency virus; HHV-6, human herpesvirus-6; IRIS, immune reconstitution inflammatory syndrome; LP, lumbar puncture; MRI, magnetic resonance imaging; n/a, not applicable; PCR, polymerase chain reaction; TB, tuberculosis; URT, upper respiratory track; V-P, ventriculoperitoneal; WCC, white cell count.

Likely HHV-6 meningoeencephalitis was defined as HHV-6 detected on FilmArray, no alternative diagnosis identified, supportive surrogate investigations, and the exclusion of chromosomally integrated HHV-6, although testing for chromosomally integrated HHV-6 in HHV-6 was not available in Botswana at the time. Possible infection was defined as HHV-6 detected on FilmArray with a clinical presentation compatible with central nervous system infection and no alternative diagnosis identified, and unlikely was defined as HHV-6 detected on FilmArray with a clear alternative diagnosis. Diagnostic structure adapted from Green DA, Pereira M, Miko B, Radmard S, Whittier S, Thakur K. Clinical significance of human herpesvirus 6 positivity on the FilmArray meningitis/encephalitis panel. Clin Infect Dis. 2018;67:24–28.

References: range: children aged under 1 month 0.15–0.35 mg/mL; children aged 1–3 months 0.3–0.8 mg/mL; infants and children over 3 months 0.5–0.9 mg/mL.

Reference range: 0.15–0.35 mg/mL.

Reference range: children aged 0–1 month 13–18 g/dL; children aged 1–2 months 10.7–17.1 g/dL; children aged 3–12 months 11.3–14.1 g/dL; men aged over 1 year 14.0-17.5 g/dL; women aged over 1 year 12.3–15.3 g/dL.

Reference range: children aged 0–1 month 32–38 0 cells/μL; children aged 1–3 months 5.0–13.5 x 10⁹/μL; children aged 3–12 months 6.0–17.5 cells/μL; adults and children aged over 1 year 4.0–11 cells/μL.
tested), and 6 were adults (of 420 adults tested; 334 adults were HIV positive and 86 HIV negative). In 652 patients, the indication for lumbar puncture was suspected central nervous system infection (CNSI), 8 patients did not have suspected CNSI, and 28 patients had complications related to a CNS device. The indication was unknown in 2 patients.

All 6 infants presented with a history of fever, 3 had seizures, 3 had respiratory symptoms, and 2 had diarrhea. No infants were HIV positive and none had a CSF pleocytosis. In 5 of the 6 infants, clear alternative clinical diagnoses were made during their admission, and they were treated for a condition that was not a CNSI. Human herpesvirus-6 meningoencephalitis was classed as unlikely to be contributing to the CNS pathology in these 5 cases. The other infant had no alternative confirmed diagnosis during admission, with possible HHV-6 encephalitis on the differential diagnosis, but had unremarkable CSF white cell count (WCC) and protein levels and recovered without receiving antimicrobial therapy. Due to the inability to perform serum HHV-6 PCR in those infants without an alternative microbiological diagnoses, we were unable to determine whether clinical presentation and CSF HHV-6 PCR detection were the result of primary systemic HHV-6 infection in these cases. All were discharged home alive.

The 12-year-old female presented to a secondary hospital with headache, neck stiffness, fever, and left-sided weakness. She was treated empirically for presumed meningoencephalitis with intravenous cefotaxime. Hydrocephalus was demonstrated on computed tomography (CT) of the brain and she was transferred to Princess Marina Hospital for ventriculoperitoneal shunt insertion. On day 21 of admission a lumbar puncture was performed for suspected shunt infection and HHV-6 was detected in the CSF. She did not receive antiviral treatment, made a clinical recovery, and was discharged home alive; HHV-6 meningoencephalitis classification was unlikely.

Among HIV-positive adults, 6 of 194 with stage 4 disease or CD4 <200 cells/mL had HHV-6 detected on FilmArray-ME compared with 0 of 140 without stage 4 disease or known CD4 counts >200 cells/µL; 0 of 86 HIV-negative adults were HHV-6 positive. Of the 6 adults with HHV-6 detected in CSF, 1 presented with a respiratory illness and was diagnosed with community-acquired pneumonia, and HHV-6 meningoencephalitis was classified as unlikely. One had a history of a known psychiatric disorder and was diagnosed with an acute psychotic episode after normal CSF WCC and protein results, normal peripheral WCC, and CT head scan; they were treated with haloperidol and discharged without receiving antiviral therapy, and HHV-6 meningoencephalitis was classified as unlikely. The remaining 4 were all determined to have CNS infections. Two had other pathogens detected in CSF in addition to HHV-6: 1 had a positive cryptococcal antigen and was treated for cryptococcal meningitis, and 1 had varicella zoster virus (VZV) detected on PCR and compatible brain magnetic resonance imaging treated with intravenous aciclovir; HHV-6 meningoencephalitis was classified as unlikely in both cases. Two had no other pathogen isolated but a presumptive diagnosis of tuberculous (TB) meningitis based on clinical presentation and laboratory findings (Table 1). Both were treated with anti-TB therapy plus antivirals for possible HHV-6 meningoencephalitis.

**DISCUSSION**

Human herpesvirus-6 was the second most commonly detected virus in the CSF of patients undergoing multiplex PCR testing for suspected CNSI in Gaborone, Botswana. Positive HHV-6 PCR results were identified in 6 infants and 1 child, none of whom were known to be HIV positive, and in 6 adults, all with advanced HIV disease. Human herpesvirus-6 was classified as being unlikely to be causing CNS pathology in 10 of 13 cases with clear alternative diagnoses, and possibly contributing to a meningoencephalitis in 3 of 13 cases, with no cases in which HHV-6 meningoencephalitis was considered the most likely diagnosis.

Primary HHV-6 infection in infants is common and typically self-limiting, and it has been associated with up to 20% of childhood admissions with fever and one third of febrile seizures in the United States [10] and Zambia [11]. With the increased availability of multiplex PCR panels, HHV-6 detection in CSF has been more frequently reported during febrile childhood illnesses [3, 5], although the clinical interpretation of these results is challenging. Human herpesvirus-6 detection in CSF has been described in children with self-limiting primary HHV-6 infection, subclinical reactivation of latent infection, chromosomally integrated HHV-6 (ciHHV-6) occurring in approximately 1% of patients after primary HHV-6 infection), and HHV-6 meningoencephalitis. One American study reported 25 HHV-6 detections in CSF from 1005 children screened with FilmArray-ME and after review of medical records, radiological findings, and ciHHV-6, it attributed 5 of these HHV-6 detections to HHV-6 meningoencephalitis [5].

In European adult populations, HHV-6 seropositivity estimates range between 78% and 92%, but there are limited data from sub-Saharan Africa. Periodically, HHV-6 can subclinically reactivate from a latent reservoir in mononuclear cells, particularly during times of host stress; detection of HHV-6 deoxyribonucleic acid (DNA) in this setting is not necessarily clinically relevant [4, 5]. However, serious infections such as encephalitis, hepatitis, uveitis, and pneumonitis can occur either as primary infection or through reactivation, predominantly in immunocompromised patients [1].

In our cohort, all 6 adults with HHV-6 DNA detected in their CSF had advanced HIV disease. There is a paucity of data on CNS HHV-6 infection in people with HIV, particularly in populations from sub-Saharan Africa. Only 18 CSF HHV-6 detections using the FilmArray-ME panel have been reported from Africa, 5 from pediatric patients with suspected meningitis in
Ethiopia where individual HIV statuses were not reported [12], and in 13 HIV-positive adult patients with suspected meningitis from 2 Ugandan studies [6, 7]. Accurately assessing the contribution of HHV-6 to disease in this population is challenging due to limited access to healthcare resources needed to support a diagnosis of HHV-6 meningoencephalitis, including neuroimaging, and the diagnostics required to reliably exclude TB meningitis or ciHHV-6. These limitations, including the lack of quantitative HHV-6 PCR in Botswana to help to exclude ciHHV-6 or primary HHV-6 infection and a lack of long-term follow-up data, restricted our ability to definitively diagnose or exclude HHV-6 meningoencephalitis.

Further complicating the clinical interpretation of HHV-6 detection in CSF in our context was the presence of coinfections, with an additional pathogen detected in the CSF of 2 adults in our study: 1 with VZV and 1 with cryptococcal meningitis. Human herpesvirus-6 was not believed to be a pathogen in either patient. Codetections of HHV-6 and other pathogens have been reported previously in sub-Saharan Africa with a 2021 Ugandan patient. Codetections of HHV-6 and other pathogens have been reported previously in sub-Saharan Africa with a 2021 Ugandan study describing 7 of 12 patients with HHV-6 detected in the CSF also diagnosed with cryptococcal meningitis [6].

CONCLUSIONS

With the expanding availability of multiplex PCR diagnostics, clinicians will increasingly have to interpret the clinical relevance of HHV-6 detection in CSF in high HIV-prevalence African settings. Our data suggest that in the majority of cases, HHV-6 detection is incidental and not contributory to CNS pathology; however, the potential role of HHV-6 as a CNS pathogen in patients with advanced HIV requires further investigation.

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Appendix 1

Pathogens detected on BioFire FilmArray-ME Panel

| Viruses | Bacteria | Yeast |
|---------|----------|-------|
| Cytomegalovirus (CMV) | *Escherichia coli* K1 | *Cryptococcus neoformans*/*Cryptococcus gattii* |
| Enterovirus | *Haemophilus influenzae* | |
| Epstein-Barr virus (EBV) This was not reported to treating clinicians results were only given to the study team retrospectively from bioMérieux after their review | *Listeria monocytogenes* | |
| Herpes simplex virus 1 (HSV-1) | *Neisseria meningitidis* | |
| Herpes simplex virus 2 (HSV-2) | *Streptococcus agalactiae* | |
| Human Herpes Virus 6 (HHV-6) | *Streptococcus pneumoniae* | |
| Human parechovirus | | |
| Varicella Zoster Virus (VZV) | | |