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Case Report

Clinical Significance of Cerebrospinal Fluid Herpesvirus 6 Positivity- A Case Series Study

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Abstract

Human herpesvirus 6 (HHV-6) is a common pathogen at childhood, remains in a latent form and can reactivate, causing encephalitis with the impairment of the immune system. The acute primary HHV-6 infection in adult immunocompetent hosts is being questioned. The cerebrospinal fluid (CSF) HHV-6 positivity can be detected in infection, in latency period, in asymptomatic reactivation or in viral chromosomic integration. To establish the clinical significance of CSF HHV-6 positivity, the authors reviewed the CSF HHV-6 positive cases in a tertiary hospital from 13 years. A total of 2111 tests were made with 0.9% of HHV-6 positivity. Only 2 cases were considered "likely" HHV-6 infected, reinforcing that most positive results do not indicate infection. Immune status and quantitative viral load studies on CSF and blood can be of great benefit, but clinical judgement is fundamental to determine the significance of HHV-6 positivity and need for treatment.

Keywords: Human herpesvirus 6; Human herpesvirus 6 chromosomally integrated; Encephalitis; HHV-6 viral load

Abbreviation: HHV-6- Human herpesvirus 6; CSF- Cerebrospinal fluid; PCR- Polymerase chain reaction; ME-Meningoencephalitis panel; MRI-Magnetic resonance imaging; CT-Computed tomography; ciHHV6-Chromosomally integrated human herpesvirus (ciHHV-6); HSCT-Hematopoietic stem-cell or solid organ transplants

1. Introduction

Human herpesvirus 6 (HHV-6) is a common pathogen from the *Herpesviridae* family and can cause meningoencephalitis [1-5]. Most adults and children have been exposed to this virus and maintain seropositivity against the HHV-6 for a lifetime [4, 5]. After the primary infection, the HHV-6 remains in a latent form and can reactivate, causing encephalitis with the impairment of the immune system, especially after bone marrow, hematopoietic stem-cell or solid organ transplants [2,6,7]. The acute primary HHV-6 infection in immunocompetent hosts is being questioned [6].

The encephalitis is an inflammatory process of the brain that is characterized by fever associated with an altered state of consciousness, seizures or neurological deficits, cerebrospinal fluid (CSF) pleocytosis and imaging and electroencephalographic changes [1, 4]. The main cause of encephalitis is viral [1], being the herpesvirus family one of the most frequent causative viruses [1, 4]. Sometimes, despite the extensive diagnostic testing, the cause of the encephalitis is not identified [4].

In the reported literature HHV-6 encephalitis has a high mortality rate and the patients who survive have rapid neurological compromise [6, 7]. Recent studies conclude that an etiological distinction based on clinical features between human viral encephalitis is not always feasible, although herpes simplex encephalitis seems to have more pronounced pleocytosis and more commonly imaging and electroencephalogram changes [1]. HHV-6 encephalitis typically affects the limbic system and manifests with symptoms consistent with this affected area: short-term memory loss, confusion, disorientation and encephalopathy [6, 7]. Magnetic resonance imaging (MRI) changes are found characteristically in the hippocampus and amygdala [7]. To establish the etiology of the encephalitis, the search for microorganisms in CSF can be selective to specific viruses or can be extended using a multiplex Polymerase chain reaction (PCR)-based detection panel which identifies the presence of multiple organisms (6 bacteria, 7 virus and 1 fungus) [1, 2]. HHV-6 is frequently identified[2] but its positivity in the CSF does not always indicate active infection because it can be detected also in the latency period, asymptomatic viral reactivation and in

viral chromosomic integration [5]. There are no validated treatments so far [7, 8]. Only foscarnet, ganciclovir and cidofovir have demonstrated efficacy in vitro and in a limited number of case reports [7, 8].

It is important to establish the diagnosis because if HHV-6 encephalitis is mistakenly affirmed, the right diagnosis can be missed. In fact, the unique feature of this virus of integrating the host's chromosomes can contribute to overestimate the diagnosis of HHV-6 encephalitis. On the other hand, if the diagnosis is not considered, unnecessary and potentially harmful exams and treatments can be done or prescribed. Thereby, it is relevant to study the clinical significance of the CSF HHV-6 positivity in immunocompetent patients with neurologic impairment. Therefore, the authors reviewed all the CSF HHV-6 positive cases in a tertiary hospital in the last years.

2. Material and Methods

This study was a single-centre retrospective transversal observational study conducted in a tertiary Hospital. The ethics committee of this centre approved the study protocol. Eligible patients were adults (aged >18 years) who were hospitalized at this hospital between January 2008 to January 2020 and who tested positive for HHV-6 in cerebrospinal fluid. In our hospital, the filmarray meningoencephalitis panel (ME panel) was only introduced in May 2019, so until this date every HHV-6 DNA search was requested by the patient's physician.

Patients' electronic records were reviewed: demographic, clinical, laboratory and imagiological data were retrieved. Afterwards, the information was evaluated by a panel of experts, composed by 2 Internal Medicine specialists and a Neurology specialist. Each specialist reviewed each patient individually and graded each case according to a likelihood scale – likely, possible, unlikely, impossible. In the event that opinions differ, a meeting between the experts was conducted so a consensus could be reached.

3. Results

During the period of the study a total of 2111 HHV-6 DNA PCR tests were made, of which 278 underwent testing with ME panel. Only 19 patients tested positive for HHV-6 in CSF (0.9%). These patients were mostly male (12) with a median age of 48 years-old and a median absolute deviation of more or less 19 years. Amongst the group only two were immunocompromised. Fever and behavioural changes were the most common presenting symptoms (11 and 8, respectively).

The first "likely" case was a 66-year-old immunocompromised woman who had two types of lymphomas (diffuse large B-cell lymphoma and a nodular sclerosis classic Hodgkin lymphoma) and underwent bone marrow autologous transplantation. Four weeks after the procedure, she initiated fever, confusion, temporal disorientation, and seizures.

Her MRI showed abnormalities evolving the limbic system at the right lobe, hippocampus and in bilateral temporo-basal regions compatible with HHV-6 encephalitis. Central nervous system neoplastic involvement was excluded with CSF cytologic evaluation and immunophenotyping. The filmarray ME panel was only positive for HHV-6 and the rest of exams were negative (JC virus DNA, CSF treponemic and non-treponemic tests, cryptococcus, bacterial and mycobacterial cultures). A plasma HHV-6 DNA test by PCR was also positive. Acyclovir was initiated but her clinical condition slowly deteriorated, and she deceased at the twelfth day of treatment.

The other "likely" case was a 58-year-old immunocompetent man with no risk factors for HHV-6 encephalitis who initiated behaviour changes and non-fluent dysphasic speech associated with sub-febrile temperature (37.5ºC). His extensive diagnostic tests only revealed the presence of HHV-6 DNA in CSF through PCR. Neither plasma HHV-6 PCR test or HHV-6 serologies were done at that time. His brain computed tomography (CT) scan and MRI were normal. The whole search for other etiologies, specifically CSF PCR tests and serologies for other human herpesvirus, was negative. The patient experienced a total recovery with antiviral therapy (acyclovir 10mg/kg every 8 hours for 15 days).

The first "possible" case was a 53-year-old immunocompetent man transferred from another hospital, where he was hospitalized for a week. He presented with complaints of anorexia, fever, and headache. CSF was positive for HHV-6 and no imagiological changes compatible with encephalitis were found. The second "possible" case was an 82-year-old immunocompetent woman, presenting with fever, seizures, behavioural changes, myalgias. CSF was positive for HHV-6, no imagiological changes were found. Both cases had no alternative explanation, despite neither being compatible with a typical HHV-6 infection. Either patient fully recovered after receiving antiviral therapy with acyclovir during 14 and 21 days, respectively.

The eleven "unlikely" patients had positive CSF for HHV-6 and had other clinical explanation. Although in these cases, we could not exclude a concomitant HHV-6 infection because each case had features compatible with HHV-6 infection. Only one patient underwent antiviral therapy (acyclovir for 8 days), and all progressed accordingly to their alternative diagnostic expected progression. The four "impossible" cases had an alternative credible diagnosis and no HHV-6 encephalitis suggestive feature.

Patient	Clinical Consensus	Age	Sex	Immunocompromissed	Presenting signs and symptons	CSF WBC (/uL)	CSF Lymphocytes (%)	Imagiological findings compatible with encephalitis (CT or MRI)	Antiviral therapy	Alternative diagnosis
1	Likely	65	Female	Yes	Fever, seizures, behavioural changes	112	0,62	Yes	Yes	No
2	Likely	58	Male	No	Fever, altered mental status, behavioural changes, speech impairment and gait ataxia	140	0,8	No	Yes	No
3	Possible	53	Male	No	Fever, headache and anorexia	730	0,92	No	Yes	Acute meningitis
4	Possible	82	Female	No	Fever, seizures, behavioural changes, memory impairment and myalgias	37	0,1	No	Yes	Viral meningoencephalitis
5	Unlikely	31	Female	No	Fever, headache, cough and upper respiratory airway symptoms	5	0	No	No	Tension headache and upper respiratory infection
6	Unlikely	45	Male	Yes	Fever, myalgias, progressive lower limbs weakness	125	0,74	No	No	HIV associated polyneuritis
7	Unlikely	27	Male	No	Fever, headache and vomits	33	0,45	No	Yes	Enteroviral meningites
8	Unlikely	80	Female	No	Fever, behavioural changes, speech impairment, left side sensitive neglect and hypoesthesia	1	0	No	No	Ischemic stroke right middle cerebral artery

9	Unlikely	26	Male	No	Fever, headache and nocturnal hyperhidrosis	1200	0	Yes	No	Subacute bacterial meningitis
10	Unlikely	83	Female	No	Behavioural changes, altered mental status and myalgias	18	0,75	No	Yes	Multiple acute ischemic strokes
11	Unlikely	26	Male	No	Diplopia and left side weakness	75	0,27	No	Yes	Multiple sclerosis
12	Unlikely	18	Male	No	Fever, cough, sore throat and progressive lower limbs weakness	4,6	0,018	No	No	Guillain-Barré syndrome
13	Unlikely	48	Female	No	Behavioural changes	0	0	No	No	Ischemic leukoencephalopathy
14	Unlikely	55	Male	No	Progressive ascending bilateral weakness and paraesthesia hands and feet	0	0	No	No	Guilain-Barré syndrome
15	Unlikely	31	Male	No	Convulsions, altered mental status and speech impairment	5	0	No	No	Epilepsy
16	Impossible	54	Male	No	Behavioural changes	1	0	No	No	Alzheimer
17	Impossible	41	Male	No	Fever, headache and other symptoms	500	0,82	No	Yes	VZV meningites
18	Impossible	56	Male	No	Fever, behavioural changes, altered mental status, speech impairment, rash, nausea and vomits	83	0	No	No	Meningoencephalitis caused by trypanossoma
19	Impossible	44	Female	No	Paraesthesia of the left hand, forearm and leg	0	0	No	No	Crytogenic stroke

Table 1: Summarizes the clinical features of each patient, CSF results, imagiological findings and definitive diagnosis.

4. Discussion

In our hospital, in accordance with previous studies [5, 9, 10], HHV-6 DNA was detected in approximately 0.9% of all CSF samples. The judgements of our expert panel reinforce the existing idea [6, 10] that most positive results for HHV-6 in CSF are not likely to indicate infection. Considering our large number of samples and extended period of time, it may be reasonable to assume that a very low percentage of our general population, like others [9], carries HHV-6 DNA chronically and asymptomatically. There is evidence that the virus circulates ubiquitously in the community with the high incidence of *exanthema subitum* and having the capacity to integrate in the human germline genome and be inherited in a mendelian manner [5, 10, 11]. Consequently, viral HHV-6 genome can always be detected in any body sample with nucleated cells of the patients who have chromosomally integrated human herpesvirus (ciHHV-6) [5, 10, 11]- in about 1% of the population [5]. Our study suggests, however, that HHV-6 can be detected even in the absence of nucleated cells (3 of our patients had no white blood cells in CSF). For this phenomenon we may consider three possible explanations: nucleated cells could have been destroyed during sample preparation; although rare, nucleated skin epithelial cells could be present in the sample [12] or another mechanism may exist, like the detection of latent virus originated from ciHHV-6 [10].

According to our expert panel, only two of 19 patients had a likely HHV-6 encephalitis. The two "likely" cases had the typical symptoms described in literature that are commonly associated with HHV-6 encephalitis such as fever, memory loss, confusion, changes of behaviour and one of the patients had also seizures [2, 6, 7]. However, only one of them had the characteristic HHV-6 MRI finding: hyperintense lesions on T2-weighted at the amygdala and hippocampus appearing as acute limbic encephalitis [2, 6, 8]. At the beginning of the infection imaging can be normal[13] and, unfortunately in the second "likely" case, it was not repeated.

Additionally, the HHV-6 encephalitis typically occurs 2-6 weeks after HSCT as we have seen in the first case [7, 8, 14]. The most likely mechanism is reactivation of HHV-6, which is more common in immunocompromised patients [5, 10, 14]. The reactivation is usually seen in patients submitted to allogenic transplant, but it is also possible after autologous stem cell transplantation as in this case [10].

In the two "possible" cases, it is difficult to assign HHV-6 as the causative organism because neither patient was immunocompromised, but once there was no alternative diagnosis, HHV-6 encephalitis was considered. In fact, HHV-6 encephalitis in immunocompetent patients is regarded as extremely rare [8, 15] and controversial [5, 6, 10]. In these patients, ciHHV-6 reactivation theory is certainly more difficult to accept and there is even scarce evidence of its existence [10]. However, there are some reported cases of HHV-6 encephalitis in immunocompetent patients [15], who experienced seroconversion during the infection. In clinical cases resembling these two, quantitative PCR in CSF and blood, HHV-6 viral load, chromosomal integration and serology can add important information to

clinical judgement. A positive qualitative detection of HHV-6 DNA may not be conclusive because it can measure ciHHV-6. Ideally, a whole blood sample should be tested at the same time and if the viral loads exceeds $>10^4$ HHV-6 DNA copies/mL and the ratio of viral and human genomes is 1:1, it is possibly a case of ciHHV-6 [5, 7]. Some studies suggest that higher levels of HHV-6 in plasma are associated with an increased risk of HHV-6 encephalitis [16, 17], still if the diagnosis does not become certain.

Even the distinction between HHV-6 species (HHV-6B and HHV-6A) may have been useful as most HHV-6 infections are due to the reactivation of HHV-6B [7, 10]. Unfortunately, in our hospital the PCR analysis is only qualitative, HHV-6 viral load and chromosomal integration is not tested, and serology was searched only in one patient. Our expert panel felt it difficult to evaluate those cases with this missing information. We shall emphasize that because of the retrospective design, the authors did not interfere in patients' clinical outcomes, treatments or exams and that the interpretation of existing information can be hampered because it is sometimes too summarized. CSF positivity for HHV-6 in the "impossible" and "unlikely" cases were not clinically significant, as these patients had other diagnosis and the evolution was consistent with the alternative diagnosis. The expert panel had very few doubts in classifying this group of patients.

In our sample, only two patients were immunocompromised, mainly because haematology patients are treated in another hospital. If they were not, our population of hematopoietic stem-cells transplanted patients and blood cancers would be larger and we could probably present more cases of HHV-6 encephalitis, as this group is particularly affected. Another high-risk group, although less often, solid organ-transplanted patients [2, 10], are also not operated or followed in our hospital.

Most of the 19 patients received no treatment, while others received acyclovir empirically, considering the most frequent causes of viral encephalitis. It was usually maintained due to favourable response. Acyclovir is not the most adequate antiviral therapy in HHV-6 encephalitis because the virus lacks thymidine kinase what makes this therapy poorly effective [8]. There are no validated treatments so far, but a more effective targeted therapy like foscarnet, cidofovir or ganciclovir [8], shall be administered as soon as possible due to poor outcome [7]. Patient immune status and once again, quantitative viral load studies on CSF and blood can help the decision to treat HHV-6 positive patients.

To sum up, our study highlights the difficulty to consider HHV-6 as the causative agent of infection for patients who test positive for HHV-6 in the CSF. False-positive results for HHV-6 DNA CSF are frequent and must be cautiously interpreted so unnecessary treatment is avoided. Typically, HHV-6 encephalitis presents as acute limbic encephalitis and CSF proteins and pleocytosis are unremarkable, especially in immunosuppressed patients who are not able to

generate a strong immune response [2, 10]. If the typical MRI changes are present the diagnosis is simplified. Additionally, in the early and in the late periods of the infection, MRI can be normal, contrarily to HSV infection which affects frequently extratemporal regions and takes longer to resolve [13], what helps to distinguish between these entities. Although quantitative viral load studies may be of great benefit, clinical judgement is still fundamental to determine the significance of HHV-6 positivity and to decide to treat these patients in proper time.

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Conflict of Interest Statement

The authors declare no conflicts of interest.

Author Contributions

IB, RMD and ASC contributed with the study concept, design and drafting of the manuscript; all authors contributed with the acquisition of data, analysis and interpretation; EFF, MJR and ASC formed the panel of experts; IB and RMD performed literature review with the supervision of ASC; all authors contributed with the critical final revision of the manuscript.

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