

Original Article

Searching the Human Herpes virus 6 and 7 (PCR) In CSF of Children Admitted In Pediatric Ward of Rasoul Hospital, Tehran, Iran

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ABSTRACT

Background and Objectives: Meningoencephalitis in Iranian children is frequent, but encephalitis is rare. The frequency of HHV-6 and HHV-7 in central nervous system diseases of our children is unclear. The aim of this study was searching the DNA-s of HHV-6 & HHV-7 in CSF samples of children with meningoencephalitis

Materials and Methods: A cross sectional study (2007-2009) was done in Pediatric Ward in Rasoul Hospital, Tehran Iran. Totally, 150 CSF samples obtained from children (1-180 months) with meningoencephalitis. Conventional and BACTEC Ped Plus medium, latex agglutination tests were used for ruling out the bacterial causes. We searched the DNA-s of HHV-6 & HHV-6 quantitatively by Real time - PCR in 150 CSF samples obtained from children with meningoencephalitis.

Results: 60.7 % of cases were male. Fever was reported (>38.5) in 74%; irritability in 70%; Convulsion was seen in 53% of cases. Herpes virus was detected in 12% (18/150) of cases. Both HHV-6 & HHV-7 were found in 6% of all cases. HHV-6 DNA was detected in 4.7% (6) and HHV-7 DNA was detected in 2 cases (1.4%) without any correlation to age, sex and clinical signs. HHV-6 was slightly more frequent than HHV-7.

Conclusion: Our data indicate that herpes viruses are not uncommon causes in children with meningoencephalitis. The incidence was lower than other references.

Keywords: Cerebrospinal Fluids; Herpes Viruses; PCR

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Introduction

Meningoencephalitis in children is frequent. But encephalitis is a rare and potentially life-threatening illness affecting the CNS characterized by an acute inflammatory process of brain. Inflammation due to viral infection and replication can occur within the brain parenchyma (encephalitis), spinal cord (myelitis), leptomeninges (meningitis), dorsal nerve roots (radiculitis) and/or nerves (neuritis) (1-3).

The role of HHV-6 and 7 in central nervous system disease is an area of ongoing investigation (4-8). Human herpes virus 6 and HHV-7 belong to the roseolovirus genus of the betaherpesvirus subfamily. Members of this genus are characterized by growth in T lymphocytes although they can also infect other cell types. Infections with either agent occur primarily during childhood (8-10). The range of CNS manifestations ascribed to these viruses includes asymptomatic infection, febrile convulsions, seizure disorders, meningitis, meningoencephalitis, facial palsy, vestibular neuritis, demyelinating disorders, hemiplegia, and rarely, fatal encephalitis (9, 10). HHV-6 has been implicated as a cause of meningitis and encephalitis in children and adults. Infectious HHV-7 is found in approximately 75% of healthy adults; most virus transmission occurs through saliva (11,12). Accurate etiological diagnosis is essential now that effective and virus-specific therapy (for example, acyclovir, gancyclovir and foscarnet) is available. Isolation of these viruses by culture of cerebrospinal fluid (CSF) gives poor results, and PCR is now the method of choice for virus detection (13-17). Some previous studies in Iran detected the role of HSV-1 in central nervous system (CNS) diseases of children (18,19).

Detection of HHV-7 sequences in CSF or Peripheral Blood Mononuclear Cells (PBMCs)

by qualitative or quantitative PCR and the absence of HHV-7 sequences in sera/plasma indicate latent infection. Viral DNA copy number for HHV-7 quantities Viral DNA was determined by real time PCR (20).

The goal of this study was to further define the role of HHV-6 and HHV-7 as causes of CNS disease in children by searching the DNA-s of HHV-6 & HHV-7 in CSF samples in children with meningoencephalitis

Materials and Methods

A cross sectional study (2007-2009) was done in Pediatric Ward in Rasoul Hospital, Tehran Iran. This study was approved by the Ethical Committee in the Research Center of Pediatric Infectious Diseases affiliates by Iran University of Medical Sciences.

Totally, 150 CSF samples obtained from children with meningoencephalitis. Conventional and BACTEC Ped Plus medium, Latex agglutination tests, were used in some cases for ruling out the bacterial diseases. Universal Bacterial PCR assay was used (1).

We searched the DNA-s of HHV-6 & HHV-6 quantitatively by Real time - PCR in 150 CSF samples obtained from children with meningoencephalitis.

HHV-6 and HHV-7 DNA quantitation

We used a part of a multiplex PCR method that detects HHV-6 and HHV-7 simultaneously, a method which uses primers that amplify a portion of the HHV-6 U67 gene and the HHV-7 U42 gene.

The reaction mixture was placed in a Real Time PCR system (Rotor Gene 6000, Corbett, Australia). Before running, the tubes hold in 95 °C for 10 min. The sample was then subjected to 5 cycles of 30 s at 95°C for denaturation; 45 s at 55°C for annealing and 45 s for elongation at 72°C; following 40 cycles of 30 s of denaturation at 95°C, 40 s of anneal-

ing at 60°C and 40 s of elongation at 72°C; and fluorescence detection on the channels Fam (Green), for HHV6 and JOE (Yellow) for HHV7 and Cyc5 (Red) for internal control, in 60° C. The primers and probe for the real-time quantitative PCR assay were designed using Gene Runner Software. The primers and the Taq Man probe were purchased from Metabion international AG (GmbH). All DNA PCR experiments were conducted with positive and negative controls for HHV6 and HHV7 as well as human β -globin primers to confirm the presence of cellular material and exclude the presence of inhibitors.

Results

60.7 % of cases were male. Age of cases was between 1-180 months. Fever (>38.5) and irritability were the most common signs (74%; 70% respectively), and convulsion was seen in 53% of cases (Table 1). CSF changes in aseptic cases are shown in Table 2.

Herpes virus was detected in 12% (18/150) cases. Both HHV-6 & HHV-7 was found in 6% of all cases. HHV-6 DNA was detected in 4.7% and HHV-7 DNA in 2 cases (1.4%) with no correlation with age, sex and clinical signs.

Table 1- Clinical manifestation in Meningoencephalitis cases

Clinical sign&symptoms	Aseptic meningoencephalitis (%)
Fever >38.5	74
Irritability	70
Stiff neck	10
Seizure	53
Loss of consciousness	7.6
Vomiting	23.5
Headache	13.5
Focal Neurologic sign	3.5
Rash/Petechia	13
Aseptic Meningitis	30

Table 2- CSF analysis in aseptic meningitis

CSF changes	Mean	Minimum	Maximum
WBC*	60	4	120
PMN (%)**	41	0	100
Lymphocyte (%)	58	0	100
Protein (mg/dl)	42.7	9	75
Glucose (mg/dl)	68	46	90

* White Blood Cells

**Polymorphonuclear

Discussion

Clinical manifestations including fever, irritability and altered mental status accompanied with seizures were very close to other studies (1-3). Detection of all type of herpes virus family in 12% of studied cases were

similar to previous studies in Iran (17,18). We agree with other authors in role of herpes family virus in meningoencephalitis diseases in children. The human herpes virus 6 (HHV-6) and human herpesvirus 7 (HHV-7) are newly described members of the herpes

virus family that produce a spectrum of CNS disease (3-6).

In the present study, HHV-6 was slightly more frequent than HHV-7. In comparison with Caserta *et al.* study which reported 3.3% in cases with meningitis, we found higher prevalence rate (4.7%) in all cases with meningoencephalitis. Indeed neither HHV-6 nor HHV-7 DNA was detected in samples from cases with encephalitis, febrile seizures, or seizure disorders (15).

Yoshikawa *et al.* detected HHV-6 DNA in the CSF of 7% patients with clinical or laboratory evidence of encephalitis but only 5 cases were pediatric patients (17).

We found HHV-7 DNA in 2 CSF specimens (1.4%) which are much lower than other reports. HHV-7 DNA was found in CSF of 8.8% to 14% of children with neurologic symptoms, in previous studies (16, 17). Our findings presumably were different from previous reports for the following reasons: differences in extraction method; differences in PCR sensitivity; differences in degree of intactness of DNA in specimens that were thawed after being frozen for prolonged periods; geographic variation; differences in patient populations and particularly differences in age groups.

Our data indicate that a herpes virus is not uncommon causes for meningoencephalitis in children. HHV-6 may occasionally cause meningitis in young infants. The incidence is lower than other references. The difference with previous studies might be due to epidemiologic and geographic variations.

HHV-6 DNA was found in a small percentage of meningitis patients (3.3%), but neither HHV-6 nor HHV-7 DNA was detected in samples from any patients with encephalitis, febrile seizures, or seizure disorders (15), in which HHV-6 DNA was found in CSF of 14.8% of children evaluated for fever, sepsis,

or seizures, in which neuro-imaging studies were abnormal in four of the nine patients, with two demonstrating focal lesions in either the parietal lobe or in the front temporal area (17-20). In Our study, HHV-6 DNA was uncommon in CSF of children (5%).

However approximately 60% to 90% of healthy adults in the USA, Europe, and Japan have antibodies to HHV-7. Children are infected by HHV-7 later than by HHV-6. Like HHV-6, it causes Roseola (Exanthem Subitum), with HHV-7 causing approximately 5% of all Roseola cases. In transplant recipients, HHV-7 infection has also been associated with Pityriasis Rosea and simultaneous infection with CMV. This has lead to reported complications that include fatal encephalitis.

Conclusion

Our data indicate that herpes viruses are common in children with meningoencephalitis. Further studies are needed to define the role of HHV-6 and HHV-7 in neurologic disorders especially in immunocompromised hosts.

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