

*Full Length Research Paper*

# Human Herpesvirus-6 and Human Herpesvirus-7 Infection in Iranian Patients with Neurological Illness

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Human herpes virus-6 (HHV-6) and HHV-7 have been implicated as causes of meningitis and encephalitis in children and adults. In this study the presence HHV-6 and HHV-7 DNA were tested in cerebrospinal fluid (CSF) sample taken from Iranian children, suffered from meningoencephalitis. From 2007 to 2009, 150 patients from Tehran with meningoencephalitis who were referred to a pediatric ward in Rasoul Akram hospital, Tehran Iran, were enrolled in the present study. Conventional and BACTEC Ped Plus medium were used in conjunction with latex agglutination test and real time PCR for detection of HHV-6 and HHV-7 DNA in clinical specimen. All type of human herpes virus DNA was detected in 12 % (18/150) cases. HHV-6 DNA was detected in 4.7% and HHV-7 DNA was detected in 2 cases (1.4%). Human herpes virus-6 and HHV-7 DNA was detected in 6% of all studied cases. HHV-6 was slightly more frequent than HHV-7. Our findings were lower than the rate of other references but were higher than the findings of previous study in Iran. This variation might be due to differences in methods, age of study cases or epidemiologic and geographic variation.

**Keywords:** Viral meningitis, Cerebrospinal fluid, CSF, Human herpesvirus-6, Human herpesvirus-7, Iran

## INTRODUCTION

Meningoencephalitis Occur frequently in children, but encephalitis which is potentially life threatening illness affecting the central nervous system (CNS) and is characterized by inflammation of the brain is somewhat rare. Fungi, bacteria and parasites are capable of inducing encephalitis; however, viruses still constitute the major cause of the disease. Among numerous viral agents that are capable of eliciting CNS inflammation. The most common pathogens that clinicians encounter are enteric viruses, arboviruses as well as members of the human herpes virus (HHV) family (Yao et al., 2009; Negrini et al., 2000; Ward et al., 2002). The role of HHV-6 and HHV-7 in CNS disease is an area of ongoing investigation (Shiley and Blumberg, 2010; Wu et al., 2008; Ward et al., 2005; Sancho-Shimizu et al., 2007).

Human herpes virus-6 and HHV-7 belongs to the roseolovirus genus of the beta herpes virus subfamily. Human herpes-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 (Schultze et al., 2004; Hall et al., 2006; Harrison et al., 2003; De Tiege et al., 2003; Ogata et al., 2008). Infections with either agent occur primarily during childhood. The range of CNS manifestations ascribed to these viruses includes asymptomatic infection, febrile convulsions, seizure disorders, meningitis, meningoencephalitis, facial palsy, vestibular neuritis, demyelinating disorders, hemiplegic, and, rarely, fatal encephalitis (De Tiege et al., 2003; Ogata et al., 2008). Accurate etiological diagnosis is essential since those effective and virus-specific therapies (for example, acyclovir, gancyclovir and foscarnet) are available. Isolation of these viruses by culture of CSF gives poor

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results, and Polymerase chain Reaction (PCR) is now the method of choice for virus detection (D'Agaro et al., 2008; Leen et al., 2006; Caserta et al., 1994; Pohl-Koppe et al., 2001). Some previous studies in Iran detected the role of HHV in CNS diseases of children (Hosseinasab et al., 2011; Tafreshi et al., 2005; Ansari et al., 2004). Ansari et al in 2004 reported that HHV-6 and HHV-7 were uncommon causes of CNS infection in children. HHV-6 may occasionally cause meningitis in young infants (Ansari et al., 2004). 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. For detection of HHV as a causative agent of the disease viral DNA copy number was determined by Real Time-PCR (RT-PCR) (Drago et al., 1997).

## MATERIALS AND METHODS

A cross sectional/prospective study from 2007 to 2009 was done in pediatric infectious ward at Rasoul Akram Hospital in Tehran. This study was approved by the Ethical Committee in the Research Center of Pediatric infectious diseases affiliated with Tehran University of Medical Sciences. As the rule in our country and Ethical Committee, all patients for Lumbar puncture (LP) would sign the paper before LP. Patient Consent was obtained.

### Data collection

Initially, a questionnaire was completed by an authorized physician for each cases (e.g. age, gender, analysis of all CSF samples, biochemical parameters, gram stain, LPA and CSF culture in both convention and Bactec medium /or universal bacterial PCR.), and the results of final diagnosis.

### Case definition

Aseptic meningoencephalitis were defined clinically as meningoencephalitis having the parameters such as at least 10 white blood cells/mm (mononuclear cell predominance); the absence of bacterial growth on culture/or negative universal bacterial PCR (if needed) in the CSF, negative latex particle agglutination test and negative direct gram stain for bacteria in CSF.

### Exclusion criteria

We excluded all cases with bacterial meningitis known as the cause of CNS manifestations (tumor, truma, Guillan Barre Syndrome, leukemia, metabolic disorders, etc) and

simple febrile convulsion with normal CSF. One hundred and fifty CSF samples were obtained from children with aseptic meningoencephalitis. The age range of patients was 1-170 months; mean =  $25.6 \pm 29$  months, 48.8% male; 51.2% female. Conventional and BACTEC Ped Plus medium; Latex agglutination tests; and in some cases universal bacterial PCR assays were used (Yao et al., 2009). HHV-6 & HHV-7 DNA was detected in CSF samples by the RT-PCR assay.

### Extraction of DNA and Master Mix preparation

DNA was extracted from 200  $\mu$ l of CSF using High pure template PCR kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The resulting DNA preparations were stored at  $-80^{\circ}\text{C}$ . For reference viruses, the infected cells were lysed in  $2\times$  lysis buffer. Negative controls were prepared by repeating the DNA preparation protocol, but CSF was replaced with distilled water and infected cells were replaced with uninfected cells. RT- PCR test was performed using HHV-6 and HHV-7 Real -TM Quant kit (Sacace Biotechnologies, Italy).

### HHV-6 and HHV-7 DNA Quantitation

Multiplex PCR method that detects HHV-6 and HHV-7 simultaneously was as applied, a method which uses primers that amplify a portion of the HHV-6 U67 gene and the HHV-7 U42 genes, the reaction mixture was placed in a RT- PCR system (Rotor Gene 6000, Corbett Research, Australia, and USA). Before start runs the tubes were held in  $95^{\circ}\text{C}$  for 10 minutes. The samples were then subjected to 5 cycles of 30s at  $95^{\circ}\text{C}$  for denaturation, 45s at  $55^{\circ}\text{C}$  for annealing and 45s for elongation at  $72^{\circ}\text{C}$ ; following 40 cycles of 30s of denaturation at  $95^{\circ}\text{C}$ , 40s of annealing at  $60^{\circ}\text{C}$  and 40s of elongation at  $72^{\circ}\text{C}$ ; and fluorescence detection on the channels Fam (Green), for HHV6 and JOE (Yellow) for HHV7 and Cyc5 (Red) for internal control in  $60^{\circ}\text{C}$ . The primers and probe for the real-time quantitative PCR assay were designed using Gene Runner Software. The primers and the TaqMan 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  $\beta$ -globin primers to confirm the presence of cellular material and exclude the presence of inhibitors.

### Statistical analysis

Quantitative variables were summarized as mean  $\pm$  standard deviation (SD) and qualitative variables as count with percentage. The Student's t test was used to

**Table 1.** Clinical Signs and symptoms in cases

Clinical Signs and symptoms	Bacterial	Aseptic
Fever	27	22
Irritability	26	21
Stiff neck	11	3
Seizure	10	8
Loss of consciousness	4	4
Vomiting	12	7
Headache	9	3
Focal Neurologic sign	3	1
Petechia	2	5

**Table 2.** CSF analysis in children with aseptic meningoencephalitis

CSF changes	Mean	Minimum	Maximum
WBC	60	4	120
PMN (%)	41	0	100
LYMPH (%)	58	0	100
PROTEIN (mg/dl)	42.7	9	75
GLUCOSE (mg/dl)	68	46	90

determine significant differences in means for all continuous variables. P value <0.05 was considered statistically significant.

## RESULTS

Clinical signs and symptoms in cases with meningoencephalitis are presented in (Table 1) Fever (>38.5) and irritability were the most common signs (74%; 70%); convulsion seen in 53% of cases.

## PCR results

Herpes viruses were detected in 12% (18/150) of cases. HHV-6 DNA detected in 4.7% (Ward et al., 2005) and HHV-7 DNA was detected in 2 cases (1.4%) with no correlation with age, sex and clinical signs.

## DISCUSSION

Clinical manifestations including Fever, irritability (70-74%), and altered mental status accompanied with seizures observed in 34% of cases are similar to earlier findings (Yao et al., 2009; Negrini et al., 2000; Ward et al., 2002). Detection of all types of Herpes virus family in 12% (18/150) of studied cases is very important and are in agreement with the previous studies in Iran

(Hosseininasab et al., 2011; Tafreshi et al., 2005). Our data are similar to the other authors in the role as herpes family virus in meningoencephalitis diseases in children. The HHV-6 and HHV-7 are newly described members of the herpesvirus family that produce a spectrum of CNS disease (Ward et al., 2002; Shiley and Blumberg, 2010; Wu et al., 2008; Ward et al., 2005).

In the present study HHV-6 infection was slightly more frequent than HHV-7. In compare with Caserta et al (1994) study which reported 3.3% in meningitis cases, we found higher prevalence rate (4.7%) in all cases with meningoencephalitis. Neither HHV-6 nor HHV-7 DNA was detected in samples from any patients with encephalitis, febrile seizures, or seizure disorders (Caserta et al., 1994). In Our study, HHV-6 DNA was uncommon in CSF of children (5%). Yoshikawa et al detected HHV-6 DNA in the CSF of 7% (9/ 138) of patients with clinical or laboratory evidence of encephalitis but only 5 cases were pediatric patients (Yoshikawa et al., 2000). We found HHV-7 DNA in 2 CSF specimens (1.4%) which are very lower than some other reports. In earlier studies conducted by Pohl-Koppe et al. (2001) and Yoshikawa et al. (2000) found HHV-7 DNA in CSF of 8.8% to 14% of children with neurologic symptoms (Pohl-Koppe et al., 2001; Yoshikawa et al., 2000). Ansari et al (2004) found HHV-6 DNA in 3 of 245 samples and HHV-7 in 0 of 245 samples of Iranian children. The three patients with HHV-6 DNA were < 2 months old. They concluded that HHV-6 and HHV-7 were uncommon causes of CNS infection in children. HHV-6

may occasionally cause meningitis in young findings adults (Ansari et al., 2004). Our data indicated that herpes viruses are not uncommon causes of meningoencephalitis in Iranian children. Our findings presumably may have differed from previous findings due to the epidemiologic and geographic variations (should added to differences in methods, differences in age of studies).

Our study has some limitations: For clinical decision and treatment, the number of cases was low. Also, we did not investigate presence of HHV-6 and HHV-7 DNAs in immunocompromised hosts with neurologic disorders.

In Conclusion, HHV-6 and HHV-7 found in 6% of all studied cases. HHV-6 was slightly more frequent than HHV-7. The incidence is slightly lower than other references. However, our data indicates that herpes viruses are not uncommon causes in children with meningoencephalitis. Our findings are lower than other references but are higher than previous study in Iran. It might due to differences in methods, age of studies cases or epidemiologic and geographic variation. Further studies are required to define the role of HHV-6 and HHV-7 in neurologic disorders especially in immunocompromised hosts.

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