Human herpesvirus 6 infection in febrile children: frequency in an Iranian referral hospital

H. FARSHADMOGHADAM*, B. POURAKBARI†, S. MAHMOUDI*, R. H. SADEGHI† and S. MAMISHI*

Department of Infectious Diseases, School of Medicine; and †Pediatric Infectious Diseases Research Center, Tehran University of Medical Sciences, Tehran, Iran

Accepted: 27 June 2014

ABSTRACT

Polymerase chain reaction (PCR) tests for virus in blood and saliva are frequently positive in persons with past infection, re-infection with new strains or latency with or without repeated reactivation of human herpesvirus 6 (HHV6). The aim of this study is to determine the frequency of HHV6 infections in children aged two years or under with an initial diagnosis of fever during an evaluation in the paediatric emergency department of the Children's Medical Center, an Iranian referral hospital, using PCR methodology. In all children, the clinical characteristics noted at the initial evaluation as well as demographic and laboratory findings were obtained. Among 150 patients (91 male, 59 female) admitted to the paediatric emergency department, HHV6 was found in 49 (33%; 14 female [29%] and 35 male [71%]). Rash was seen in 14/49 (29%) of HHV6-positive cases, while 35 cases without rash had a positive PCR test (71%). Seizures were found in 78/150 (52%) patients. There was no significant association between seizures and positive HHV6 results (43% in patients without seizure; 57% in cases that developed seizure). Although standard PCR on samples including blood cannot discriminate between latent and active HHV6 infection, nearly a third of patients (mainly children less than one year old) had HHV6 infection.

KEY WORDS: Child. Herpesvirus 6, human. Infant. Polymerase chain reaction.

Introduction

Human herpesvirus 6 (HHV6) has been associated with febrile convulsions, particularly prolonged afebrile and febrile seizures.[1–3] Exanthem subitum (roseola) was first described by Zahorsky in 1913,[4] and in 1988 Yamanishi *et al*.[5] reported that HHV6 is the cause of the disease.

Roseola normally involves fever (39–40°C) for two to four days (may last up to seven days), followed by a rash lasting from a few hours to four days.[5]

Similar to other members of the herpesvirus family, HHV6 can persist in the host with the establishment of latent infection.[6] Polymerase chain reaction (PCR) tests for virus in blood and saliva are frequently positive in persons with past infection, re-infection with new strains of the virus, or latency with or without repeated reactivation of HHV6 virus.[6,7]

The aim of this study is to determine the frequency of HHV6 infections in children aged two years or under with an initial diagnosis of fever during an evaluation in the paediatric emergency department of the Children's Medical Center, an Iranian referral hospital.

Materials and methods

Children aged ≤2 years with an initial diagnosis of fever during an evaluation in the paediatric emergency department were enrolled in the study. The study period covered June 2011 to May 2012, during which 150 children fulfilled the enrollment criteria. The case definition of a febrile convulsion was a seizure in infants and children in association with a fever of 38°C or higher, but without evidence of any definitive causative disease.

Blood samples were collected during the acute phase (in the presence of fever) following informed consent obtained from parents of the children after the project had been explained in detail. Clinical characteristics were noted at initial evaluation and demographic and laboratory findings were obtained from medical records.

Samples of DNA from blood were extracted using the AccuPrep Genomic DNA Extraction Kit (Bioneer, Korea), following the manufacturer's instructions. A PCR method for HHV6 was performed on extracted blood samples using two primers: 397F (5′-TCG AAA TAAGCA TTA ATA GGC ACA CT-3′) and 493R (5′-CGG AGT TAA GGC ATT GGT TGA-3′).[8] The 50-µL reaction mixture contained 10 mmol/L Tris (pH 8.6), 500 nmol/L $MgCl_2$, 50 mmol/L KCl, a 300 mmol/L concentration of each deoxynucleoside triphosphate, a 0.6 µmol/L concentration of each primer, and 1.5 units *Thermus aquaticus* (*Taq*) polymerase (Fermentase).

All PCR reactions were completed with standard controls to prevent contamination, including dedicated equipment and geographic separation of pre-amplification and post-amplification reactions. A PCR reaction mixture without added DNA was used as a negative control in all experiments. The amplification procedure was conducted under identical conditions as follows: 94°C for 6 min, then 94°C for 30 sec, 53°C for 30 sec and 72°C for 45 sec for 40 cycles, and finally 72°C for 7 min.

Correspondence to: Dr. Setareh Mamishi
Department of Pediatric Infectious Diseases
Children's Medical Center Hospital, School of Medicine
Tehran University of Medical Sciences
No.62, Gharib Street, Keshavarz Blvd., Tehran, Iran
Email: smamishi@sina.tums.ac.ir

British Journal of Biomedical Science 2014 71 (3)

Table 1. Laboratory findings in children ≤2 years with and without HHV-6 infection.

	HHV6 negative		HHV6 positive	
	Mean±SD	95% CI	Mean±SD	95% CI
Temperature	38.15±0.61	37.9–38.3	38.34±0.58	38.1–38.5
PMN (%)	49.0±1.7	44.5–53.5	47.0±1.8	41.6–53.8
Lymphocyte (%)	40.5±1.6	36.1–44.8	38.6±1.8	32.5–44.7
ESR	20.3±1.5	16.3–24.4	22.7±2.0	16.0–29.5
CSF protein	32.3±3.7	22.5–42.0	20±1.2	16.7–25.0
CSF glucose	62.4±1.1	59.3–65.5	60.2±1.4	55.6–64.9

PMN: polymorphonuclear leucocytes; ESR: erythrocyte sedimentation rate; CSF: cerebrospinal fluid

Statistics

Comparison between groups was made using unpaired Student's *t*-test or two-tailed Fisher's exact test.

Results

Among 150 patients (91 male, 59 female) admitted to the paediatric emergency department of the Children's Medical Center, an Iranian referral hospital, HHV6 was found in 49 (33%; 14 female [29%] and 35 male [71%]; $P=0.06$). Mean age of cases with primary HHV6 infection was 9.11 months. HHV6 infection was found in 20/49 (41%) cases with acute fever in children aged less than six months, 15/49 (31%) of children aged 6–12 months, and 14/49 (29%) children aged over one year.

Rash developed in 29/150 (19%) patients, and 14 (74%) had HHV6 in their blood. Rash was found in 14/49 (29%) cases with positive HHV6, while 35 (71%) cases without rash had a positive PCR test ($P=0.046$).

Seizures were found in 78/150 (52%) patients. There was no significant association between seizures and positive HHV6 results (43% in patients without seizure and 57% in cases who developed seizure [$P>0.05$]).

Laboratory findings are shown in Table 1.

Discussion

Clinical characteristics of primary infection with HHV6 have been well described and consist primarily of a febrile illness in infants, with seizure as the key complication.[9] It has been reported that persistent HHV6 infection is common in children.[10] In addition, there have been reports of HHV6 DNA in serum and/or cerebrospinal fluid (CSF) or by virus isolation in control individuals.[11–14] Álvarez-Lafuente *et al*.[14] found HHV6 DNA in the peripheral blood mononuclear cells of 53.4% patients with multiple sclerosis and 30.4% healthy blood donors.

In the present study, the frequency of HHV6 infection was 33% and the majority occurred in patients aged less than one year. Primary infection with HHV6 causes acute febrile illness, usually in children aged between six months and one year,[15] and HHV6 infection accounts for 20% of all cases of acute fever in children in this age group.[9,16] Some studies have reported that the majority of HHV6B infections occur between the ages of six months and 24 months.[1,16,17]

In another study, undertaken during 2003–2004, among 1591 HHV6 PCR samples from various sources, including blood, CSF, ascitis and tissue biopsy, only 43 (3%) samples were positive.[18] In a study by Caserta *et al*.,[19] 29 (11.6%) children with primary HHV6 infection were identified out of 250 children ages ≤3 years old, and, as in the present study, all children were febrile (mean temperature 39.8°C) and there was no difference in degree of fever or frequency of rash between the groups.

Febrile seizures were identified in approximately 10% children with primary HHV6 infection, and 10–20% febrile seizure cases occurred in children aged under two years.[9,15,20–22] In the present study, 57% patients with HHV6 infection had febrile seizure, rash was seen in 19%, while 71% cases without rash had a positive PCR test. In the study by Asano *et al*.[15] primary HHV6 infection was observed in 94 males and 82 females, and macular or papular rash appeared in 98% (on face or trunk, or both).[15]

In conclusion, although standard PCR on different samples including blood cannot discriminate between latent and active HHV6 infection, in the present study approximately a third of patients, mainly children aged less than one year, had HHV6 infection. □

References

1 Ansari A, Weinberg A. Epidemiology of herpes viruses 6 and 7 in children. *Perspect Med Virol* 2006; **13**: 37–50.

2 Juntunen A, Herrgård E, Mannonen L *et al*. A major role of viruses in convulsive status epilepticus in children: a prospective study of 22 children. *Eur J Pediatr* 2001; **160** (1): 37–42.

3 Sugaya N, Yoshikawa T, Miura M, Ishizuka T, Kawakami C, Asano Y. Influenza encephalopathy associated with infection with human herpesvirus 6 and/or human herpesvirus 7. *Clin infect Dis* 2002; **34** (4): 461–6.

4 Zahorsky J. Roseola infantum. *Arch Pediatr* 1954; **71** (4): 124–8.

5 Yamanishi K, Okuno T, Shiraki K *et al*. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* 1988; **1** (8594): 1065–7.

6 Pass RF. HHV6 and HHV7: persistence and vertical transmission. *J Pediatr* 2004; **145** (4): 432–5.

7 Zerr DM, Huang M-L, Corey L, Erickson M, Parker HL, Frenkel LM. Sensitive method for detection of human herpesviruses 6 and 7 in saliva collected in field studies. *J Clin Microbiol* 2000; **38** (5): 1981–3.

8 Collot S, Petit B, Bordessoule D *et al*. Real-time PCR for quantification of human herpesvirus 6 DNA from lymph nodes and saliva. *J Clin Microbiol* 2002; **40** (7): 2445–51.

9 Hall CB, Long CE, Schnabel KC *et al*. Human herpesvirus-6 infection in children – A prospective study of complications and reactivation. *N Engl J Med* 1994; **331** (7): 432–8.

10 Caserta MT, McDermott MP, Dewhurst S *et al*. Human herpesvirus 6 (HHV6) DNA persistence and reactivation in healthy children. *J Pediatr* 2004; **145** (4): 478?84.

11 Chapenko S, Millers A, Nora Z, Logina I, Kukaine R, Murovska M. Correlation between HHV-6 reactivation and multiple sclerosis disease activity. *J Med Virol* 2003; **69** (1): 111–7.

12 Akhyani N, Berti R, Brennan MB *et al*. Tissue distribution and variant characterization of human herpesvirus (HHV)-6: increased prevalence of HHV-6A in patients with multiple sclerosis. *J Infect Dis* 2000; **182** (5): 1321–5.

13 Knox KK, Brewer JH, Henry JM, Harrington DJ, Carrigan DR. Human herpesvirus 6 and multiple sclerosis: systemic active infections in patients with early disease. *Clin Infect Dis* 2000; **31** (4): 894–903.

14 Alvarez-Lafuente R, Martin-Estefania C, de las Heras V *et al*. Active human herpesvirus 6 infection in patients with multiple sclerosis. *Arch Neurol* 2002; **59** (6): 929–33.

15 Asano Y, Yoshikawa T, Suga S *et al*. Clinical features of infants with primary human herpesvirus 6 infection (exanthem subitum, roseola infantum). *Pediatrics* 1994; **93** (1): 104–8.

16 De Bolle L, Naesens L, De Clercq E. Update on human herpesvirus 6 biology, clinical features, and therapy. *Clin Microbiol Rev* 2005; **18** (1): 217–45.

17 Abdel-Haq NM, Asmar BI. Human herpesvirus 6 (HHV6) infection. *Indian J Pediatr* 2004; **71** (1): 89–96.

18 Revest M, Minjolle S, Veyer D, Lagathu G, Michelet C, Colimon R. Detection of HHV-6 in over a thousand samples: new types of infection revealed by an analysis of positive results. *J Clin Virol* 2011; **51** (1): 20–4.

19 Caserta MT, Hall CB, Schnabel K, Long CE, D'Heron N. Primary human herpesvirus 7 infection: a comparison of human herpesvirus 7 and human herpesvirus 6 infections in children. *J Pediatr* 1998; **133** (3): 386–9.

20 Barone SR, Kaplan MH, Krilov LR. Human herpesvirus-6 infection in children with first febrile seizures. *J Pediatr* 1995; **127** (1): 95–7.

21 Kondo K, Nagafuji H, Hata A, Tomomori C, Yamanishi K. Association of human herpesvirus 6 infection of the central nervous system with recurrence of febrile convulsions. *J Infect Dis* 1993; **167** (5): 1197–200.

22 Ward K, Gray J. Primary human herpesvirus-6 infection is frequently overlooked as a cause of febrile fits in young children. *J Med Virol* 1994; **42** (2): 119–23.