Iranian Journal of Microbiology
Volume 6 Number 2 (April 2014) 87-90

Prevalence of HHV-6 in cerebrospinal fluid of children younger than 2 years of age with febrile convulsion

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Received: May 2013, **Accepted:** December 2013.

ABSTRACT

Background and Objectives: Febrile convulsion is a common disorder in children. Viral infections such as human herpes virus 6 (HHV-6) which results in roseola infantum may contribute to developing seizure. The objective of this study was to determine the prevalence of HHV-6 by detecting DNA in cerebrospinal fluid (CSF) of children with febrile convulsion and without any rash of roseola infantum.

Materials and Methods: In this descriptive cross-sectional study, CSF of 100 children younger than 2 years of age with febrile convulsion was evaluated for detecting HHV-6 DNA by PCR. All of them were referred to emergency ward in Pediatric Medical Center from March 2010 to March 2011. General information, clinical manifestations, laboratory tests and outcomes were collected in the questionnaires.

Results: One hundred children including 59 males and 41 females were evaluated. HHV-6 was detected from CSF in six patients (6%) by PCR. Mean age was 8 months old. All children were younger than 12 months old. The most common primary manifestation was fever alone. None of them had rash. Majority of cases occurred in winter. All patients recovered without any encephalitis.

Conclusion: These findings showed that primary infection with HHV-6 is frequently associated with febrile convulsion in infants which may be at risk for subsequent development of epilepsy.

Keywords: Human Herpes Virus 6, Febrile Convulsion, Children, CSF

INTRODUCTION

Febrile convulsion is the most common type of seizure in children between 3-60 months old (1-2). Its frequency is 2-5% (3). In recent years, multiple investigations have been conducted to evaluate the role of viral infections in developing febrile convulsion (4). Among viral infections, HHV-6 has been considered as a probable cause of febrile convulsion and its prevalence evaluated in various areas in the world (4-7).

Roseola infantum or exanthem subitum is a common infection due to HHV-6 in the infants. Classic form of roseola infantum is high grade fever that lasts for 3-5 days and maculopapular rash that appears subsequent -ly after subsiding fever. This classic manifestation only appears in 15% of patients and mostly nonspecific manifestations such as fever, rhinorrhea, cough, seizure, irritability, lymphadenopathy and nausea develop (6-8). Previous studies showed that febrile convulsion is common during exanthem subitum (9). For many years, investigators believed that fever is the cause of seizure in this infection, but recent studies have shown that HHV-6 is able to involve the central nervous system during roseola disease (10, 11).

The aim of this research was to determine the prevalence of HHV-6 in children with febrile

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convulsion using PCR method for detection of HHV-6 DNA in cerebrospinal fluid (CSF).

MATERIALS AND METHODS

Sample collection. In this descriptive cross sectional study, 100 children younger than 2 years of age with febrile convulsion were enrolled. They were referred to emergency ward in Pediatric Medical Center. After obtaining informed consent from parents, cerebrospinal fluid was obtained from all of them and evaluated for analysis and detecting HHV-6 by PCR method in Virology Department of Tehran University of Medical Sciences.

Molecular assays. DNA was extracted from 150 µL of samples using the Roche extraction kit according to the manufacturer's instruction. Primers designed based on conserved part of U22 gene of HHV-6 genome. Forward primer: 5´-TCGAAATAAGCATTAATAGGCACACT-3´and Reverse primer:

5´- CGGAGTTAAGGCATTGGTTGA-3´. Each 50 µl PCR mixture contained 5 µl of 10X PCR buffer, 2 µl Mgcl2 50 mM, 1 µl dNTPs 10mM, 2.5 µl of each of the primers with 10pmol/ µl concentration, 0.4 µl of Taq DNA polymerase, 31.5 µl ddH2O and 5 µl of extracted DNA. The amplification process was conducted under following conditions: 94˚C for 6 min; then 40 cycles of 94˚C for 30s, 55˚C for 30s, and 72˚C for 45s; and finally 72˚C for 7 min. Positive control was purchased from Vircell company.

Statistical method. General information and laboratory findings were collected in questionnaires and finally data were analyzed by SPSS-13.

RESULTS

100 children younger than 2 years old were evaluated. They had referred to Pediatric Medical Center with fever and convulsion. All of them were undergone lumbar puncture for ruling out meningitis and encephalitis. HHV-6 was detected from CSF in six patients (6%) by PCR method. General information and laboratory findings on blood and CSF in all studied patients as well as HHV-6 positive patients have been shown in Table1.

Fifteen percent of patients had history of previous seizure. Six patients (6%) had seizure about 48 hours before fever. Seven patients (7%) had vaccination about 48 hours before febrile convulsion including six cases with MMR vaccination and one child with influenza vaccination. Two cases (2%) had rash at the time of febrile convulsion. Gram positive diplococi was found in CSF Gram staining in only one case.

In Five patients (83.4%) with roseola, seizure was generalized tonic clonic whereas 1 case (16.6%) had atonic seizure. In the patients with HHV-6 positive all were younger than 12 months old and the youngest patient was 4 months old. Exanthem subitus (classic rash for roseola infantum) was not manifested in the patients.

DISCUSSION

HHV-6 infection mostly occurs in early years of life. It's most common manifestations are fever, rash and seizure (6). HHV-6 primary infection is associated with 10-20% of febrile convulsions in infants. Most of them did not experience rash. HHV-6 DNA was detected in CSF of 6% children and adults with focal encephalitis with unknown origin (6).

In this study HHV-6 DNA was detected from 6% of CSF specimens obtained from 100 children younger than 2 years old who were referred to Pediatric Medical Center. The results were similar to several investigations in various areas worldwide. For instance, Ward and coworkers studied 200 children younger than 2 years old with primary HHV-6 infection. They detected HHV-6 DNA from CSF in 7% of cases (6). Of course, in some studies the prevalence of HHV-6 has been lower or higher than this study. For example , in Tavakoli & Hull study in 2007, prevalence of HHV-6 DNA positivity in 1482 CSF samples obtained from cases with meningitis or encephalitis was reported to be 1.75% (7). Also, in some investigations such as Bertolani study, 35% of patients with febrile convulsion were positive for HHV-6 (12). Similar to other investigations, none of the patients with febrile convulsion and HHV-6 had exanthema subitus. The results of this research were comparable with other studies which showed the peak acquisition of primary HHV6 infection, from 6 to 15 months of age. Before 6 months of age, there was a low rate (<10%) of primary HHV6 infection (6).

In present study, mean of WBC was 12000 cell/ mL with a relative lymphocytosis. One of studied cases has significant poleocytosis with predominacy of lymphocyte in CSF. In Roseola, WBC counts of

Table 1. General information and laboratory findings of all studied patients.

variables		All children with febrile convulsion (n = 100)	Children with HHV-6 PCR positive (n = 6)
Gender	Male	59 (59%)	6 (100%)
	Female	41 (41%)	0 (0%)
Mean of age		11 months	8 months
Season	Spring	15 (15%)	0 (0%)
	Summer	5 (5%)	1 (16.6%)
	Fall	43 (43%)	2 (33.3%)
	Winter	37 (37%)	3 (50%)
Primary manifestations	Fever	98 (98%)	6 (100%)
	Fever and diarrhea	13 (13%)	0 (0%)
	Fever and vomiting	8 (8%)	0 (0%)
	Fever and cough	19 (19%)	1 (16.6%)
	Fever and coryza	14 (14%)	1 (16.6%)
Episode of seizure	One episode	81 (81%)	5 (83.3%)
	More than one episode	19 (19%)	1 (16.6%)
Interval between fever and convulsion	Less than 24 hours	87 (87%)	5 (83.3%)
	More than 24 hours	13 (13%)	1 (16.6%)
Laboratory findings	WBC	16679 ± 11880 (cell/mm3)	11986 ± 8842 (cell/mm3)
	PMN	52.1 ± 19.3(%)	45 ± 7(%)
	Lymphocyte	41.1 ± 26.7(%)	47 ± 7(%)
	ESR	77 ± 16 (mm/h)	16 ± 6 (mm/h)
CSF analysis	WBC (mean ± SD)	130 ± 27 (cell/mm3)	
	RBC (mean ± SD)	3450 ± 616 (cell/ µl)	
	Sugar (mean ± SD)	62 ± 17 (mg/dl)	57 ± 7 (mg/dl)
	protein (mean ± SD)	22 ± 30 (mg/dl)	22 ± 14 (mg/dl)
Encephalitis		0 (0%)	0 (0%)
Complete recovery		100 (100%)	6 (100%)

8000 – 9000 cell/ml may be found during the first few days of fever in children. The CSF in children with HHV6 associated febrile seizures typically is normal. The CSF from rare cases of HHV6 associated meningoencephalitis and encephalitis is characterized by a mild pleocytosis with predominance of mononuclear cells, normal glucose and normal to slightly elevated protein (6).

In Yoshikawa study in 2009, 86 cases with exanthema subitum and encephalitis/encephalopathy were evaluated and HHV-6 DNA was detected in 21 patients (13).

In present study all of our patients survived and we had no death whereas in Yoshikawa study 2 cases of death occurred among 86 cases (13). In another study on 138 patients with encephalitis, 9 cases were HHV-6 positive which four of them had complete recovery, 3 cases had neurological complications, one case had status epilepticus and one case died (14).

Suga and colleagues evaluated 21 children with HHV-6 infection and neurological complications (seizure and encephalitis). In their study HHV-6 DNA was detected in CSF by PCR method in 3 cases. One of them died immediately after admission and seizure was remained in one case as a complication (15).

Finally this study was a cross sectional descriptive study on immunocompetent children.

The results of our investigation would help us to decide about applying molecular tests as a diagnostic method in pediatric febrile convulsion. Also, the results on these expectations would help us to use antibiotics rationally in the febrile convulsion.

According to this study and the prevalence of roseola infantum in the children with febrile convulsion

evaluation of CSF (detecting HHV-6 DNA by PCR) is recommended in children younger than 12 months old who admitted with febrile convulsion.

Further studies on immunocompromised children are recommended for comparing results in immunocompetent and immunocompromised children. Because our sample size was small, more studies with larger number of participants are recommended for achieving more accurate results. Also, regarding to low frequency of bacterial meningitis in febrile convulsion cases, it is recommended to avoid irrational prescribed antibiotics.

In conclusion these findings showed that primary infection with HHV-6 is frequently associated with febrile convulsion in infants which may be a risk factor for subsequent development of epilepsy.

ACKNOWLEDGEMENT

The authors thank the entire staff of the National Influenza Center, School of Public Health, Tehran University of Medical Sciences.

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