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Research Article

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Detection of human herpesvirus 6 DNA in cerebrospinal fluid of children with neurological disorders in Damascus, Syria

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ABSTRACT

Febrile convulsion is a common disorder in children. Viral infections such as human herpes virus 6 (HHV-6) which results in roseolainfantum may contribute to developing seizure. The objective of this study was to test cerebrospinal fluid (CSF) for the detection of HHV-6 DNA in a group of children in Damascus with febrile convulsion of whom bacterial involvement had been ruled out. In this cross-sectional study, CSF from90 children between 7 month-8 years of age with neurological disorders characterized by febrile convulsion was evaluated for detecting HHV-6 DNA by PCR. All of them were referred to emergency department in Damascus Hospital or Alassad University Hospital from December 2013 to February 2015. Ninety children including 48 males and 42 females were enrolled in the study. HHV-6 DNA was detected from CSF in three patients (3.3%) by PCR. All the HHV-6 DNA positive children were younger than two years. The most common primary manifestations were fever and convulsion. None of them had rash. These findings showed that primary infection with HHV-6 is frequently associated with febrile convulsion in children less than two years old and detecting the HHV-6 DNA by PCR is recommended in those children, for initiating the proper antiviral therapy.

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

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INTRODUCTION

Febrile convulsion is the most common type of seizure in children between the ages of 3 months and 5 years (1). It is estimated that about one in every 25 children will have at least one febrile seizure, and more than one-third of these children will have additional febrile seizures before they outgrow the tendency to have them (2).

There have been many investigations on the role of viruses in developing febrile convulsions (3). Among viral infections, HHV-6 has been considered as a probable cause of febrile convulsion and its prevalence has been studied indifferent parts of the world (3-5).

Human herpesvirus 6 (HHV-6) is a member of *Herpesviridae*(6). There are two variants, HHV-6A and HHV-6B (7). Clinical disease caused by HHV-6A is not well understood. HHV-6B causes exanthema subitum (roseolainfantum) (8), febrile illness, febrile seizures, or fever with otitis media. Roseolainfantum or exanthem subitum is a common infection due to HHV-6 in the infants and is characterized by a high fever and the development of a rash after the fever has resolved. Itis estimated that only 15% of patients develop these classic symptoms and other nonspecific manifestations such as fever, rhinorrhea, cough, seizure, irritability, lymphadenopathy and nausea usually develop (5-9). HHV-6 has been associated with a variety of neurological complications including encephalitis (10,11) meningitis (12) and febrile convulsions (13).

The detection of HHV-6 infection in children with neurological disorders, in Syria, has not been previously described. The aim, therefore, of this study was to determine the presence of HHV-6 DNA in cerebrospinal fluid (CSF) samples from a group of children in Damascus, with neurological disorders characterized by febrile convulsion using PCR and compare the results to those of other countries.

EXPERIMENTAL SECTION

Study Design: Prospective cross sectional study

Sample collection:
The study included90 children aged 7 months- 8 years with neurological disorders characterized by febrile convulsion. They were referred to the emergency department in Damascus Hospital and Alasad University Hospital between December 2013 and February 2015. There were 48 males and 42 females. The CSF samples were obtained from patients for whom CSF specimens were taken to investigate meningo-encephalitis illness. The samples were kept at -70°C until analysis. The specimens were tested for the presence of HHV-6 by PCR method in the Faculty of Pharmacy research laboratory, Damascus University.

Materials and methods
DNA was extracted from the CSF samples using GF-1 Viral Nucleic Acid Extraction Kit (Vivantis, Malaysia) according to the manufacturer's instructions. For the detection of HHV6 DNA, the primer pair: H6-6 (5`AAGCTTGCACAATGCCAAAAAACAG3`) and H6-7 (5`CTCGAGTATGCCGAGACCCCTAATC3`) (14) that amplify 223 bp of ORF 67 were used. The reaction mixture contained 10 mMTris/HCl pH 8.3, 50 mMKCl, 1.5 mM MgCl2, 0.001% w/v gelatin, 200 mMdNTPs, 0.2 mM of each primer, 1.25 U Amplitaq and5 µl sample DNA in a total volume of 50 µl. Cycling conditions were an initial ''hot-start'' denaturing step (1 cycle) of 94°C/12 min, 55°C/30 sec, 72°C/30 sec followed by 40 cycles of 94°C/30 sec, 55°C/30 sec, 72°C/30 sec. PCR products were run on 2% Agarose gel and were then visualized by staining in ethidium bromide and viewed under UV light.

RESULTS AND DISCUSSION

The study included 90 children aged 7months-8 years. The mean age was 32 months. HHV-6 DNA was detected from CSF in three patients (3.3%) by PCR method (Figure 1). General information and laboratory findings of all the patients including the HHV-6 DNA positive cases are shown in Table1.Bacterial culture was negative in all of the cases. The White Blood Cells count in the positive cases ranged from 140-400 cell/ml. Fever was documented in all of the positive and negative cases. The three HHV-6 positive patients were all younger than 18 months. Exanthemsubitus (classic rash for roseolainfantum) was not manifested in any of the patients including the positive ones. All the 90 patients had febrile convulsion, 8 had loss of consciousness, 4 diarrhea, and 16 vomiting. Among HHV-6-positive patients, one had loss of consciousness, two vomiting and two diarrhea. All of the positive cases were boys.

Table 1. Clinical and laboratory findings of the study population

Variable	All patients (n=90)	Children with positive HHV-6 PCR (n=3)
Gender		
Males	48	3
Females	42	0
Mean age (month)	32 months	13
Primary manifestations		
Fever	90	3
Fever and convulsion	90	3
Fever and vomiting	16	2
Fever and diarrhea	4	2
Loss of consciousness	8	1
CSF analysis		
WBC: cell/ml (range)	87-1720	80-1150

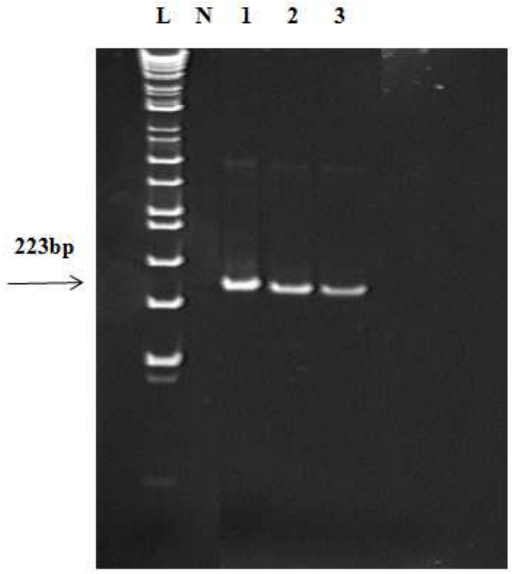

Figure1. Detection of HHV-6 DNA in CSF samples from three children with neurological disorders
L-ladder, C- negative control, lanes 1-3: positive cases

DISCUSSION

HHV-6 infection mostly occurs in early years of life. It's most common manifestations are fever, rash and seizure (9). About 10-20% of primary infections with HHV-6 are associated with of febrile convulsions in infants. The majority of them with no documented rash. HHV-6 DNA was detected in CSF of 6% children and adults with focal encephalitis with unknown origin (9).

In the current study, HHV-6 DNA was detected by PCR in CSF specimens of **3 (3.3%) of 90** children with neurological disorders characterized by fever and convulsions.

Febrile convulsions due to HHV-6 infection has been reported by several studies. In some of them, patients completely recovered, and in some others, neurologic sequel and death were reported. The results of the current study were similar to several investigations in different parts of the world.

For instance, HHV-6 DNA was detected from CSF in 7% of the 200 children less than two year old who had documented primary HHV-6 infection (15). In a similar recent study, Setareh Mamishi *et al* reported the detection of HHV-6 DNA from 6% of CSF specimens obtained from 100 children younger than 2 years old who were referred to Pediatric Medical Center for febrile convulsion (16). Noorbakhsh S *et al* also detected HHV-6 DNA in CSF samples from 9(6%) out of 150 children with meningoencephalitis (17)

A similar but lower finding was reported by Tavakoli & Hull who showed a prevalence of HHV-6 positivity of1.75% in 1482 CSF samples obtained from cases with meningitis or encephalitis (18).Similar to our findings, Jila Yavarian detected HHV-6 in 10 (8.8%), of a total of 114 patients with suspected encephalitis, 90% of which were boys with mean age 7.7 months (19)

Other studies revealed higher prevalence of HHV-6 DNA in neurological disorders.

Yoshikawa *et al*. tested 86 cases of exanthema subitum with encephalopathy for the presence of HHV-6 DNA. They detected the viral DNA in21(24%) of the cases; two of them were fatal (20). In another study HHV-6 DNA was demonstrated in CSF from 9 (42%) out of the 21 patients with encephalitis and neurological disorders (21).

One of the limitations of the current study is the small sample size. We recommend further studies with larger number of cases including immunocompromised children for comparing the results between the immunocompetent

and immunocompromised children. We also suggest including other common viruses such as enteroviruses and herpes simplex virus to assess the coinfection and other possible agents involved in febrile convulsion in children.

CONCLUSION

This study has identified for the first time the presence of HHV-6 DNA in the CSF samples from a group of children population in Damascus with febrile convulsions, and has indicated that detecting the HHV-6 DNA by PCR is recommended in children less than two years old with neurological disorders, for initiating the proper antiviral therapy.

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