Arch Pediatr Infect Dis. 2018 October; 6(4):e63954. doi: 10.5812/pedinfect.63954.
Published online 2018 July 30. **Research Article**

Evaluation of Viral (HHV6, Adenovirus, HSV1, Enterovirus) and Bacterial Infection in Children with Febrile Convulsion by Serum PCR and Blood Culture Mofid Children's Hospital , 2016 - 2017

Abdollah Karimi [1], Mohammad Sakhavi [1], Negin Nahanmoghaddam [1], Farideh Shiva [1], Leila Azimi [1], Mehdi Shirdust [1], Shahnaz Armin [1,*] and Ahmad Reza Shamshiri [2]

[1] Pediatric Infections Research Center, Research Institute for Children Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[*] *Corresponding author*: Associated Professor, Pediatric Infections Research Center, Mofid Children's hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: arminsh_2000@yahoo.com

Received 2017 November 11; **Revised** 2018 March 30; **Accepted** 2018 April 10.

Abstract

Background: Febrile seizures (FS) are common in young children. Viral infections that result in high fever are frequent etiologic agents that lead to febrile seizures. Human Herpesvirus 6 (HHV6), Influenza virus, adenovirus, and enterovirus have been named as the most common viruses causing high fevers in young children. Although bacterial infections have rarely been found as causative agents, many children with febrile seizures are treated with empiric antibiotics.
Objectives: To determine the epidemiology of the viral (HHV6, Adenovirus, HSV1, Enterovirus) and bacterial infection in children presenting with febrile seizures.
Methods: In a descriptive study, data was collected from 93 children, 3 months to 5 years of age, hospitalized with febrile seizures between September 2016 and April 2017. Relevant information was documented on a previously designed questionnaire. Blood samples were collected for culture, Serum 16S rRNA for bacteria, and PCR assay for 4 viruses (HHV6, adenovirus, enterovirus and HSV), in addition to routine investigations.
Results: Of the 93 patients, serum PCR results were negative for 73 patients (78.49%) and 15 (16.13%) were positive for viruses (11 for HHV6 and 4 for Adenovirus). Serum 16S rRNA for bacteria was detected in 5 cases and blood culture was positive in 4 cases.
Conclusions: Findings of this study indicate a significant prevalence of viruses and a very low rate of bacterial infection in children with febrile seizures, thus, negating the use of empirical antibiotic therapy.

Keywords: Febrile Seizure, Simple FS, Complex FS, Viral Infections, Bacterial Infection

1. Background

Febrile seizures are among the most common seizure disorders leading to hospitalization in children and have been reported as the most common type of seizures in Iranian children (1-6).

A total of 2% - 5% of children have experienced at least one febrile seizure before they reach the age of 5 (1, 7, 8).

The pathophysiology of FS is still unclear; it is likely that it is an age-related response of the immature brain to fever in children with a genetic predisposition, with a multi-factorial mode of inheritance, a high concordance rate in monozygotic twins, and a higher prevalence in first- and second-degree relatives (5, 8-11). Studies in Iran have shown a significant geographical variance in the prevalence of febrile seizures in different parts of the country (2).

In addition to high fever, risk factors for FS include infections, recent immunization, and previous or family history of FS (1, 5, 9-12). Although a proprietary virus has not been identified as the cause of FS, Influenza A virus, HHV6, adenovirus, enterovirus have been implicated as the most common agents that provoke a febrile convulsion (1, 5, 9, 13, 14). Viral infections may trigger a seizure not only due to high fever but release of inflammatory cytokines especially IL-1B that provoke epileptogenesis, as has been shown in animal studies (13, 15). In addition, some viruses may cause direct neuroinvasion as HHV-6 DNA has been detected in the cerebrospinal fluid of some children with FS (16).

In contrast, bacterial infections are less common, and studies have revealed a low risk of bacteremia in well-appearing children with febrile seizures in populations where children have received the *H. Influenza* type B (Hib)

vaccine (17, 18).

Various methods, like cultures or PCR assays on nasophryngeal aspirates, serum samples, or cerebrospinal fluid have been used to isolate microorganisms associated with FS; in the recent years the 16S ribosomal RNA gene (16S rRNA gene) has been sequenced to identify unknown microorganisms in different diseases (19).

The present study was conducted in Mofid Children's Hospital to determine the epidemiology of infectious agents associated with febrile seizures in children by PCR assay on serum specimens collected from the patients. Due to the financial resources limitation, only blood samples were evaluated in this study. We choose them based on prevalence reports in other studies.

2. Objectives

To determine the epidemiology of the viral (HHV6, Adenovirus , HSV1, Enterovirus) and bacterial infection in children presenting with febrile seizures.

3. Methods

Inclusion criteria (1, 20):

- A seizure associated with an elevated temperature greater than 38°C

- A child older than three months and younger than six years of age

- Absence of central nervous system infection or inflammation

- Absence of acute systemic metabolic abnormality that may produce convulsions

- No history of previous afebrile seizures

All children, between the ages of 3 months and 5 years, hospitalized during the study period (September 2016 to April 2017) with a diagnosis of febrile seizures, were included in the study.

For sample size calculation we used the binomial distribution formula ("accepting a population prevalence as not exceeding a specified value"). If only 1 positive sample is found among our study population with a sample size of 93, we can accept with 95% confidence that the frequency of that agent is not exceeding 5%.

- type of study:

Descriptive cross sectional study

- the population study:

Children aged 3 months to 5 years with F.C. referred to the Mofid Children's Hospital in 2016-2017.

- Data collection techniques:

Questionnaire and Paraclinical tests

(CBC-diff ESR , CRP , B/C, U/C , Serum PCR for four Viruses and Serum 16s rRNA PCR)

A questionnaire was designed to document relevant data from the study subjects. After obtaining written parental consent for the study, the following information was recorded: age, gender, type of seizure, associated symptoms (Cough, coryza, diarrhea, vomiting, etc.), body temperature, time elapsed between onset of fever and the seizure, drug consumption, and family history of seizures. In addition to routine investigations (CBC, ESR, CRP, CSF, if indicated), blood samples were collected for culture and also for PCR assay (serum 16S rRNA for bacterial infection and specific primers to identify HHV6, Adenovirus, Enterovirus and HSV1).

Blood samples containing EDTA were sent to the Laboratory of the Infectious Disease Research Center under standard conditions within a maximum period of 18 hours after admission. Samples were divided into two equal volumes upon receipt, one for extraction of bacteria and another for the extraction of viruses.

Extraction of bacterial DNA was done using the High-Clean PCR Template Preparation Kit (Roche, REF. No. 11796828001). The proliferation of the 16srRNA gene was done by using specific primers to identify the bacterial infection (21).

The second series of blood samples were taken using the QiAmp Dsp virus spin kit (QIAGEN. REF. No. 61704) extraction kit that simultaneously isolates all DNA and RNA viruses. DNA HHV6, Adenovirus, and HSV1 have been detected by PCR and specific primers for identification. cDNA synthesis has been done for enterovirus before identification of PCR because it is a RNA virus. Primers are depicted in Table 1. PCR conditions have been described previously (22-25).

3.1. Statistical Methods

We reported categorical variables as counts and percentages by SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). To calculate 95% confidence intervals for frequency of viral infections, the Wilson score interval method was used.

4. Results

Overall, 93 patients were included in the study; 54 patients (58.1%) were male. As the study period stretched from September to April, most samples were collected in the fall, winter, and early spring (Table 2).

Clinical characteristics of patients are presented in Table 3. Most patients (89%) were less than 36 months of age and more than 63% were between 12 and 36 months old (Table 3). Less than 10% had a past history of previous seizures, however, more than 31% had a positive family history of FS (Table 3).

Table 1. Primer Sequences Used For Detection of Microorganisms

Primer	Sequence (5'→3')	Reference
16 S rRNA		21
F	AGAGTTTGATCCTGGCTCAG	
R	GGTTACCTTGTTACGACTT	
Entero virus		22
F	CAAGCACTTCTGTTTCCCCGG	
R	ATTGTCACCATAAGCAGCCA	
HHV6		23
F	GGTGCTGAGTGATCAGTTTC	
R	TTCTCCAGATGTGCCAGGGA	
HSV 1		24
F	TTTTCTGCTCCAGGCGGACT	
R	AGCGTCTTGTCATTGGCGA	
Adenovirus		25
F	GCCGAGAAGGGCGTGCGCAGGTA	
R	TACGCCAACTCCGCCCACGCGCGCT	

Four patients (4.3%) had a positive blood culture and 16 S rRNA was positive for bacteria in 5 cases (5.4%) (Table 3).

Of the 93 patients, serum PCR results were negative for 73 patients (78.49%); 15 (16.13%) were positive for viruses and 5 for bacteria. Of the 15 patients with positive serum PCR for viruses, 11 (11.8%) were positive for HHV6, and 4 (4.3%) for Adenovirus (Table 4).

A total of 24 patients (25.8%) had symptoms of URI (runny nose, cough, and nasal congestion). In comparison with patients without URI symptoms, 6 (25%) cases had positive serum PCR for the viruses in first group.

Another 24 patients (25.8%) were presented with gastrointestinal symptoms like diarrhea and vomiting. Viral PCR in patients with or without gastrointestinal manifestation was compared, it was positive in 2 (8.3%) cases in first group, while almost half of the patients were presented with fever without localizing signs.

All patients with a positive PCR had been presented with a simple febrile seizure.

Table 2. Season of Sample Collection (N = 93) and Number of Samples Positive for Virus, Mofid Hospital, 2016 - 2017[a]

Season	Samples	Positive Serum PCR
Summer	6 (6.45)	0
Autumn	56 (60.2)	8 (14.3)
Winter	29 (31.2)	6 (20.7)
Spring	2 (2.1)	1 (50)

[a]Values are presented as No. (%).

Table 3. Clinical and Laboratory Characteristics of Patients Admitted with FS (n = 93), Mofid Hospital, 2016 - 2017

Characteristics	No. (%)
Age group	
< 12 mo	24 (25.8)
12 - 36 mo	59 (63.4)
> 36 mo	10 (10.7)
Temp. at admission	
< 38°C	28 (30)
38 - 39°C	54 (58)
> 39°C	11 (11.8)
Positive past history of FS	9 (9.7)
Positive family history of FS	29 (31.2)
Associated signs/symptoms	
Respiratory infection	24 (25.8)
Gastroenteritis	24 (25.8)
None	45 (48.4)
Complex FS	18 (19.3)
Blood culture positive	4 (4.3)
Urine culture positive	4 (4.3)
16S rRNA positive for bacteria	5 (5.37)
PCR assay positive for viruses	15 (16.12)

Abbreviation: FS, febrile seizure.

Table 4. Serum PCR Results For Viruses (n = 93), Mofid Hospital, 2016 - 2017

Virus Detected in Serum	No. (%)	95%CI[a]
HHV-6	11 (11.8)	6.73 - 19.95
Adenovirus	4 (4.3)	1.69 - 10.54
HSV1	0	0 - 3.97
Enterovirus	0	0 - 3.97

[a]95% confidence interval (was calculated by Wilson score interval method).

5. Discussion

Although, generally the virus has not been identified as an etiology for FC, various studies have revealed different viruses as the cause of FC, HHV6, Adenovirus, Influenza, Enterovirus, HSV, Parainfluenza, RSV, EBV, CMV. HHV-6 is the virus most frequently associated with F.C in the US and Europe.

In our study, we found that HHV6 was the most commune viruse in this group of patients; it was compatible with other studies. Another virus identified as an etiology

F.C is adenovirus; it is also one of the viral causative agents of the F.C in most articles. We couldn't detect enterovirus, which may have been due to unequal sampling during one year. Most specimens, 85 (91.3%) patients, were taken in the autumn and winter, while enteroviruses are more common in the summer.

A total of 4 (4.3%) patients had positive blood culture; this finding is consistent with other articles that describe bacterial infections as rare causes of F.C. Therefore, with the above findings, physician shouldn't start empiric antibiotic therapy.

In the present study, evidence of viral infection was found in the serum of less than 17% of children hospitalized with febrile seizures. These figures are in stark contrast to several other studies, notably a study in Australia, in which out of 143 nasal samples that were tested for viruses, at least 1 virus was identified in 71% and viral co-infection in 34% (1). The Australian study was carried out from May 2012 to October 2013, that period in the Southern hemisphere would include one summer season, two autumns, and two winters as regards the seasonal prevalence of respiratory tract and gastrointestinal infections, while our relatively short study period was from September to April and included fall, winter, and early spring with only a few days of the summer season. In their study, rhinovirus, adenovirus, and enterovirus were the most commonly detected viruses, while the most common viral infection detected in the serum of our patients was due to HHV-6; none were caused by enterovirus. One reason for our low yield could be due to the relatively short duration of the study, also due to the fact that our study did not stretch into the summers when enterovirus infections are more frequent. In the Australian study, samples were collected from nasopharyngeal aspirates, which harbor various microorganisms, as multiple viruses were identified in most of their samples, while we used the very specific method of PCR assay of serum specimens. The researchers in Australia also identified influenza in 13% of their samples; we did not look for the influenza virus. In Asia, the influenza virus has been found to be associated with febrile seizures in the influenza season and may be accompanied by a concurrent HHV infection (1, 14). In Europe and the United States, however, HHV-6 is associated with one-third of all first-time febrile seizures in infants and children less than 2 years of age (14).

In a study from Hong Kong, nasopharyngeal aspirates collected from 923 children with febrile seizures that were collected for antigen testing of 5 common viruses over a period of 5 years, detected viral antigens in 34.4%, with influenza virus in 17.6%, adenovirus in 6.8%, followed by parainfluenza, 6%, RSV 2.7%, and Rotavirus 1.3%. HHV-6 testing was not done in the study, however, 2.9% of their children were presented with the clinical spectrum of roseola infantum, thus, making it the 4th common cause of fever in their patients (9). The low rate of viral detection in our study may also be attributed to the short duration and the small numbers as compared to the mentioned study. Although our figures for detection of viruses are relatively low, a breakdown of the figures, according to the season, reveals a higher yield of >20% in the winter season, when respiratory infections are common (Table 2).

Approximately, our patients were presented with complex febrile seizures, which is similar to some studies (9, 10, 26), but less than the reported rate of one third of all febrile seizures in other large studies (1, 8). No specific viral infection was identified in these patients and all children in whom PCR assay for viruses or bacteria was positive had been presented with simple febrile seizures.

Our figures are different from an Iranian study based on an analytical review of 21 studies on 4599 children with FS in Iran, which revealed the infectious causes as upper respiratory infection in 42.3% of cases, followed by gastroenteritis in 21.5%, otitis media 15.2%, pneumonia 8.7%, urinary tract infection 3.2%, and roseola infantum 2%, with an unidentified source in 2.8% (2). In other studies as well respiratory tract infections were found as the most common cause of FS (9, 10, 26).

Findings of this study indicate a significant prevalence of viral infections and a very low rate of bacterial infection in children with febrile seizures, thus, negating the use of empirical antibiotic therapy.

The present study underscores the need for larger studies for longer durations, sample collection from various sites, and utilization of PCR assays, which include most of the common viruses seen in children.

5.1. Limitations and Suggestions

- Use of appropriate kits: Due to the fact that enteroviruses have very different subtypes, it will be useful to use the kits that identify most of these subtypes.

- Sampling from various sites: Only blood samples were used in this study. While in most similar studies, several different sites, such as oral and nasal discharge samples, stool, and urine specimens are used.

- Examination of other viral agents causes F.C.

- Use a large number of samples.

- Use of different methods to identify infectious agent causative F.C, such as serology.

- Sampling in the four seasons of the year.

Especially, as no samples were collected during late spring or during the summer season, a period when enterovirus infections are more frequent.

- Design other study and Evaluate bacterial infection in other site such as lung, urine, stool in special cases.

Acknowledgments

This study was supported by a grant No. 8836 from the Shahid Beheshti University of Medical Sciences, Medical School, Tehran, Iran.

References

1. Francis JR, Richmond P, Robins C, Lindsay K, Levy A, Effler PV, et al. An observational study of febrile seizures: the importance of viral infection and immunization. *BMC Pediatr*. 2016;**16**(1):202. doi: 10.1186/s12887-016-0740-5. [PubMed: 27914475]. [PubMed Central: PMC5135752].
2. Delpisheh A, Veisani Y, Sayehmiri K, Fayyazi A. Febrile seizures: etiology, prevalence, and geographical variation. *Iran J Child Neurol*. 2014;**8**(3):30–7. [PubMed: 25143771]. [PubMed Central: PMC4135278].
3. Auvin S, Antonios M, Benoist G, Dommergues MA, Corrard F, Gajdos V, et al. [Evaluating a child after a febrile seizure: Insights on three important issues]. *Arch Pediatr*. 2017;**24**(11):1137–46. French. doi: 10.1016/j.arcped.2017.08.018. [PubMed: 28965695].
4. Sharawat IK, Singh J, Dawman L, Singh A. Evaluation of Risk Factors Associated with First Episode Febrile Seizure. *J Clin Diagn Res*. 2016;**10**(5):SC10–3. doi: 10.7860/JCDR/2016/18635.7853. [PubMed: 27437319]. [PubMed Central: PMC4948495].
5. Camfield P, Camfield C. Febrile seizures and genetic epilepsy with febrile seizures plus (GEFS+). *Epileptic Disord*. 2015;**17**(2):124–33. doi: 10.1684/epd.2015.0737. [PubMed: 25917466].
6. Fetveit A. Assessment of febrile seizures in children. *Eur J Pediatr*. 2008;**167**(1):17–27. doi: 10.1007/s00431-007-0577-x. [PubMed: 17768636].
7. Huang MC, Huang CC, Thomas K. Febrile convulsions: development and validation of a questionnaire to measure parental knowledge, attitudes, concerns and practices. *J Formos Med Assoc*. 2006;**105**(1):38–48. doi: 10.1016/S0929-6646(09)60107-8. [PubMed: 16440069].
8. Kimia AA, Bachur RG, Torres A, Harper MB. Febrile seizures: emergency medicine perspective. *Curr Opin Pediatr*. 2015;**27**(3):292–7. doi: 10.1097/MOP.0000000000000220. [PubMed: 25944308].
9. Chung B, Wong V. Relationship between five common viruses and febrile seizure in children. *Arch Dis Child*. 2007;**92**(7):589–93. doi: 10.1136/adc.2006.110221. [PubMed: 17284480]. [PubMed Central: PMC2083759].
10. Esmaili Gourabi H, Bidabadi E, Cheraghalipour F, Aarabi Y, Salamat F. Febrile seizure: demographic features and causative factors. *Iran J Child Neurol*. 2012;**6**(4):33–7. [PubMed: 24665278]. [PubMed Central: PMC3943022].
11. Saghazadeh A, Mastrangelo M, Rezaei N. Genetic background of febrile seizures. *Rev Neurosci*. 2014;**25**(1):129–61. doi: 10.1515/revneuro-2013-0053. [PubMed: 24399675].
12. Demicheli V, Rivetti A, Debalini MG, Di Pietrantonj C. Vaccines for measles, mumps and rubella in children. *Cochrane Database Syst Rev*. 2012;(2). CD004407. doi: 10.1002/14651858.CD004407.pub3. [PubMed: 22336803].
13. Epstein LG, Shinnar S, Hesdorffer DC, Nordli DR, Hamidullah A, Benn EK, et al. Human herpesvirus 6 and 7 in febrile status epilepticus: the FEBSTAT study. *Epilepsia*. 2012;**53**(9):1481–8. doi: 10.1111/j.1528-1167.2012.03542.x. [PubMed: 22954016]. [PubMed Central: PMC3442944].
14. Tang J, Yan W, Li Y, Zhang B, Gu Q. Relationship between common viral upper respiratory tract infections and febrile seizures in children from Suzhou, China. *J Child Neurol*. 2014;**29**(10):1327–32. doi: 10.1177/0883073813515074. [PubMed: 24453149].
15. Millichap JG, Millichap JJ. Role of viral infections in the etiology of febrile seizures. *Pediatr Neurol*. 2006;**35**(3):165–72. doi: 10.1016/j.pediatrneurol.2006.06.004. [PubMed: 16939854].
16. Mamishi S, Kamrani L, Mohammadpour M, Yavarian J. Prevalence of HHV-6 in cerebrospinal fluid of children younger than 2 years of age with febrile convulsion. *Iran J Microbiol*. 2014;**6**(2):87–90. [PubMed: 25705358]. [PubMed Central: PMC4281666].
17. Shah SS, Alpern ER, Zwerling L, Reid JR, McGowan KL, Bell LM. Low risk of bacteremia in children with febrile seizures. *Arch Pediatr Adolesc Med*. 2002;**156**(5):469–72. [PubMed: 11980552].
18. Trainor JL, Hampers LC, Krug SE, Listernick R. Children with first-time simple febrile seizures are at low risk of serious bacterial illness. *Acad Emerg Med*. 2001;**8**(8):781–7. [PubMed: 11483452].
19. Clarridge JE 3rd. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin Microbiol Rev*. 2004;**17**(4):840–62. table of contents. doi: 10.1128/CMR.17.4.840-862.2004. [PubMed: 15489351]. [PubMed Central: PMC523561].
20. Jones T, Jacobsen SJ. Childhood febrile seizures: overview and implications. *Int J Med Sci*. 2007;**4**(2):110–4. [PubMed: 17479160]. [PubMed Central: PMC1852399].
21. Chen YL, Lee CC, Lin YL, Yin KM, Ho CL, Liu T. Obtaining long 16S rDNA sequences using multiple primers and its application on dioxin-containing samples. *BMC Bioinformatics*. 2015;**16 Suppl 18**. S13. doi: 10.1186/1471-2105-16-S18-S13. [PubMed: 26681335]. [PubMed Central: PMC4682383].
22. Hong J, Kang B, Kim A, Hwang S, Lee S, Kim J, et al. Enhanced detection of enteroviruses in clinical samples by reverse transcription-PCR using complementary locked primer technology. *J Clin Microbiol*. 2010;**48**(2):615–6. doi: 10.1128/JCM.01790-09. [PubMed: 19940056]. [PubMed Central: PMC2815621].
23. Bai X, Hosler G, Rogers BB, Dawson DB, Scheuermann RH. Quantitative polymerase chain reaction for human herpesvirus diagnosis and measurement of Epstein-Barr virus burden in posttransplant lymphoproliferative disorder. *Clin Chem*. 1997;**43**(10):1843–9. [PubMed: 9342002].
24. Knezevic A, Martic J, Stanojevic M, Jankovic S, Nedeljkovic J, Nikolic L, et al. Disseminated neonatal herpes caused by herpes simplex virus types 1 and 2. *Emerg Infect Dis*. 2007;**13**(2):302–4. doi: 10.3201/eid1302.060907. [PubMed: 17479897]. [PubMed Central: PMC2725876].
25. Xie L, Yu XF, Sun Z, Yang XH, Huang RJ, Wang J, et al. Two adenovirus serotype 3 outbreaks associated with febrile respiratory disease and pharyngoconjunctival fever in children under 15 years of age in Hangzhou, China, during 2011. *J Clin Microbiol*. 2012;**50**(6):1879–88. doi: 10.1128/JCM.06523-11. [PubMed: 22442311]. [PubMed Central: PMC3372113].
26. Hussain S, Tarar SH, Moin Ud Din S. Febrile seizrues: demographic, clinical and etiological profile of children admitted with febrile seizures in a tertiary care hospital. *J Pak Med Assoc*. 2015;**65**(9):1008–10. [PubMed: 26338751].