ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/341276943

Detection of Human Herpes Virus-6 in Children

Article *in* International Journal of Psychosocial Rehabilitation · May 2020

CITATIONS
0

READS
85

1 author:

Zeytoon Alkhafaji
University of Babylon- Medicine college
16 PUBLICATIONS **2** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Put your best research and work done. View project

Corona Virus Affected of Human and Animal: A Review View project

All content following this page was uploaded by Zeytoon Alkhafaji on 10 May 2020.

The user has requested enhancement of the downloaded file.

International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 05, 2020
ISSN: 1475-7192

Detection of Human Herpes Virus-6 in Children Suffering from Febrile Convulsion

Jwan A. Ali*, Zaytoon A.R. Al-Kafaji and Adnan H. Al-Jothery

Abstract--- *A total of (150) blood samples were taken from children who suffering from febrile convulsion, during the period from April (2019) to October (2019), who admitted to Babel Hospital, Al-Noor Hospital and Al-Imam Al-Sadiq Center, at age range from six month to five years old. out of (150) blood samples, (50) samples of children who suffering from febrile convulsion fit as group one [31 male (62%) and 19 female (38%)], (50) samples of children who suffering from fever without fit as group two [34 male (68%) and 16 female (32%)] and (50) samples of control case (healthy children) as group three [male 36 (72%) and 14 female (28%).Demographic data of patients and control (age, gender, weight and residence) were studied from all blood samples were taken from children. The mean of ages for the three study groups was investigated, it was found that, there were no significant statistical relationship between them (P= 0.88), Distribution of study groups according to gender was studied, the results showed that, They were no important variances among study groups (P= 0.56) regarding gender. According to study patient's weight, there were no important statistical relationship among the study groups (P= 0.28).In this study, distribution of patient groups according to the residence was studied, it was found that, there were highly significant differences (P= 0.000) in the urban within the health group compared with patients group. Human Herpes Virus 6 was were detection from blood samples by qPCR technique. It was found that, from 50(100%) samples of conventional febrile (fit with fever), 18(36%) isolated were related of HHV6. In group of fever, It was found that, from 50(100%) samples, 12(24%) isolated were related of this virus.*

Aim of Study: *To detection of human herpes virus-6 among children suffering from febrile convulsion by qPCR technique.*

Keywords--- *Febrile Convulsion, Human Herpes Virus-6, fit with Fever, PCR.*

I. INTRODUCTION

Human herpesvirus-6 (HHV-6) is ubiquitous beta-herpes virus widely disseminated in the overall population. They primary infection commonly happens in the early years of life and residues latent in the host of the lifelong period [1]. Human herpes virus-6 is the communal collective name for Human beta herpes virus 6A (HHV-6A) and human beta herpes virus 6B (HHV-6B). These closely connected viruses are two of the nine herpes viruses known to have humans as their primary host [2]. HHV-6 infection is generally acquired very early in life, among 6 months and 2 years of age, following the loss of protective maternal antibodies. At previously period of life, infection congenital following intrauterine transmission have been report of 1% from children, infection congenital is the base related for HHV-6 in mothers [3].Human Herpesvirus-6 is the causative factor of the popular childhood contagious disease, can cause complications of the central nervous system (CNS), containing febrile seizures and encephalitis/

Jwan A. Ali, University of Babylon, Collage of Medicine, Microbiology Department, Iraq. E-mail: Jwanalhamawandi@gmail.com*
Zaytoon A.R. Al-Kafaji, University of Babylon, Collage of Medicine, Microbiology Department, Iraq.
Adnan H. Al-Jothery, University of Babylon, Collage of Medicine- Pediatric Department, Iraq.

DOI: 10.37200/IJPR/V24I5/PR202010
Received: 25 Feb 2020 | Revised: 20 Mar 2020 | Accepted: 30 Mar 2020

encephalopathy. There is too speculation that the direct inroad of the virus in to the CNS might function a crucial part in cause the complications neurological [4].In children, fever is usually a sign of infection, and fever due to other causes counting malignancy is rare [5]. The prognosis of the utmost popular forms of infections pediatric is generally excellent; these infections are much extra possible to be viral (pharyngitis, laryngitis, bronchitis, bronchiolitis, rhinitis, gastroenteritis, exanthemas) than bacterial (pneumonia, sinusitis, urinary tract infections, otitis, tonsillitis) [6, 7]. HHV-6 infection might participate to the pathogenesis of sclerosis multiple. This virus migth diagnosed via viral culture, polymerase chain reaction or serology [8]. Thus HIV too infects T-cells via the CD4 molecule, the interactions of the viruses in T-cells through the course of AIDS are significant region of investigation [9].

II. MATERIALS AND METHODS

Patients and clinical specimens

A total of (150) blood samples were taken from children who suffering from febrile convulsion, during the period from April 2019 to October 2019, who admitted Babel Hospital, Al-Noor Hospital and Al- Imam Al-Sadiq Center, at age range from six month to five years old. Any child aged from 6-60 months developed fit with fever with normal S. Na, S.Ca (ionized fraction).Four ml of blood samples were collected, and divided in to three groups 50 samples of children who suffering from febrile conversion fit, 50 samples of children who suffering from fever without fit and 50 samples as control case (healthy children). These samples were collected according to diagnosis of seiner doctors. Each blood samples were divided into two tubes (2ml putted in EDTA tube, to obtain of holly blood, and 2ml putted in gully tube to obtain serum). All samples were stored in freezing at (-20C^{o}) until used.

Control groups

Control group 1: Child matched age and sex with fever without fit.

Control group 2: Child matched age and sex without fever and fit.

Ethical approval

The ethical necessary agreement of ethical committee from Hospitals and patients followers have to obtained. furthermore, whole subjects participatory in the work are informed and the approval wished for act the experiments and publication of the data is obtained of each one prior the aggregate of samples.

Exclusion criteria

1. Age less than 6 months and more 60 months.
2. Any child with known case of epilepsy.
3. Any child with neurodevelopmental delay (CP).
4. Any child with explainable cause of FS (meningitis).
5. Any child with abnormal S.b Na, S.Ca (ionized fraction).

Genomic viral DNA Extraction

Genomic DNA were extracted for samples blood via utilizing gSYAN DNA kit extraction kit (Frozen Blood protocol) Gene aid. USA., and done according to company instructions. The extracted blood genomic DNA was

DOI: 10.37200/IJPR/V24I5/PR202010
Received: 25 Feb 2020 | Revised: 20 Mar 2020 | Accepted: 30 Mar 2020

checked via utilizing Nano drop spectrophotomater (THERMO. USA), which measured DNA concentration (ng/µL) and check the DNA purity through determination the absorbance at (260 /280 nm).

Real-Time Polymerase Chain Reaction

Real-Time PCR technique was performed for in detection of HHV-6 based amplification of major capsid protein (*MCP*) gene.

Table 1: Primers: HHV-6 PCR primers

Primer	Sequence (5'-3')		Product size	Reference
HHV-6	F	GATACATTCGCCGCAACCAC	542pb	[10]
	R	GGTGAGCTGGGATCGGTATG		

III. RESULTS AND DISCUSSION

A total of (150) blood samples were taken from children who suffering from febrile convulsion, during the period from April (2019) to October (2019), who admitted to Babel Hospital, Al-Noor Hospital and Al- Imam Al-Sadiq Center, at age range from six month to five years old. Out of (150) blood samples were taken from children, and divided in to three groups, at age range from six months to five years old. From (150) blood samples, (50) samples of children who suffering from febrile convulsion fit as group one [31 male (62%) and 19 female (38%)], (50) samples of children who suffering from fever without fit as group two [34 male (68%) and 16 female (32%)] and (50) samples of control case (healthy children) as group three [male 36 (72%) and 14 female (28%), as shown in Figure (1).The results of present study were in agreement with results obtained by [11] who found that from (300) children were collected, 150(50%) suffering from febrile convulsion fit, 75(25%) samples of children who suffering from fever without fit and 75(25%) samples as control. Shibeeb and Altufaily, [12] found that, highly significant differences in residence that taken from children who suffering from febrile convulsion (p=0.001). A study of [13] found that, all 6–month- to children 6-year-old of the diagnosis of febrile convulsion, (81.8%) of the children had febrile seizure. Febrile seizure is the utmost communal kind of convulsive disorder and one of the utmost prevalent cause of emergency hospital admission in children [14].Demographic data of patients and control (age, gender, weight and residence) were studied from all blood samples were taken from children. The mean of ages for the three study groups was investigated, it was found that, there were no significant statistical relationship between them (P= 0.88).The results of current study showed no significant difference of age on the development of fibrial convulsion and these results were a similar results that reported in the studies of [15] who found that, there was no significant differences in mean of ages between study groups. Distribution of study groups according to gender was studied, the results showed that, There were no important variances among study groups (P= 0.56). The results in this study were similar results obtained by [16] who found that, there were no important differences among children suffering from febrile convulsion regarding gender. Contrary to the results of the study, the mentioned results of [17] was seems that, febrile seizures were more common in male children over age 6 months. According to study patient's weight, there were no important statistical relationship among the study groups (P= 0.28).These results were agreement with results obtained by [18] who showed that, no important variances among study groups according to weight. In this study, distribution of patient groups according to the residence was studied, it was found that, there were highly

DOI: 10.37200/IJPR/V24I5/PR202010
Received: 25 Feb 2020 | Revised: 20 Mar 2020 | Accepted: 30 Mar 2020

significant differences (P= 0.000) in the urban within the health group compared with patients group, as shown in Table (3-1). The results in this study which similar to study obtained by [12] who found that, highly significant differences in residence that taken from children who suffering from febrile convulsion (p=0.001), all results of demographic data were shown in Table (2).Glauser *et al*., [19] found that, there was no important affect of age and sex on the kind of febrile seizure (p value=.008). In addition, the results in this study were agreement with results obtained by [20] who found that, there were no significant differences in ages between the cases and control (p=0.64).On the other hand, Heydarian *et al*., [21] was found that, the mean difference between the study groups of febrile convulsions in sex was not significant (P = 0.06). In another study of [22] who demonstrated that, febrile children were not significant difference according to gender. Some variables was deranged in small proportions like body weight and gestational age, so their statistical analysis of association cannot be reliable. Utmost of the other considered variables appeared no consequences on the characteristics of febrile seizures [23].

Table 2: Comparison of the studied patients and control groups according to their demographic data

Variables	Group 1 N=50	Group 2 N=50	Group 3 N=50	P-value
Age (months) (mean±SE)	21.26±2.72	22.96±2.56	21.64±2.30	0.88
Gender (n %) **Male** **Female**	 31(62.0) 19(38.0)	 34(68.0) 16(32.0)	 36(72.0) 14(28.0)	 0.56
Weight (kg)(mean ±SE)	9.80±0.51	10.78±0.51	9.82±0.45	0.28
Residence (n %) **Urban** **Rural**	 24(48.0) 26(52.0)	 24(48.0) 26(52.0)	 50(100.0) 0(0.0)	 0.000**

**** P value is of highly statistical significant, Group1= fever with fit, Group2= fever without fit, Group 3= healthy control, Kg= kilogram, SE= standard errors.**

Human Herpes Virus 6 was detection from blood samples by qPCR technique. It was found that, from 50(100%) samples of conventional febrile (fit with fever), 18(36%) isolated were related of HHV6,in group of fever, it was found that, from 50(100%) samples, 12(24%) isolated were related of HHV6. These results were shown in Figure (2) and Table (3).

Table 3: Comparison of the studied groups according to the result of PCR testing of HHV6

PCR test	Group 1 N=50	Group 2 N=50	Group 3 N=50	P-value			
				All Groups	G1vsG2	G1vsG3	G2vsG3
HHV6 **Positive** **Negative**	 **18(36.0%)** **32(64.0%)**	 **12(24.0%)** **38(76.0%)**	 **0(0.0%)** **50(100%)**	 **0.000****	 **0.190**	 **0.000****	 **0.000****

*** P value is of statistical significant,** P value is of highly statistical significant**

 # significant group by post hoc tests for one- way ANOVA,Group1= fever with fit, Group2= fever without fit, Group 3= healthy control, PCR= polymerase chain reaction, HHV6= human herpes virus 6

The results in this study were agreement with results obtained by [24]who found that, primary human herpes virus 6 infection is acquired mainly through the primary life to 5 years of life, and is often related with febrile seizures. Farshadmoghadam, *et al*., [25] found that HHV6 were detected among children who suffering from febrile convulsion fit in (36%) among study group. Moreover current results not agreement with the children suffering from fever only (41%) in the same study. Current results were in agreement with [15] who found that HHV6 were

DOI: 10.37200/IJPR/V24I5/PR202010
Received: 25 Feb 2020 | Revised: 20 Mar 2020 | Accepted: 30 Mar 2020

detected among children who suffering from febrile convulsion fit in (43.4%) among study group. The primary HHV-6 infection didn't reveal raised risk for recurrent febrile convulsions [26].Oikawa *et al*., [27] find that, children that aged of 6 months to 5 years without known neurologic disease, were examined for primary HHV-6 infection, via real-time polymerase chain reaction in acute-phase plasma and via indirect immunofluorescent assay for antibody titers in acute and convalescent serum. Out of (65) children contaning in the analysis, (55) experienced the initial febrile convulsion. Other study of find that, out of 10 to 55 children for a initial febrile (18%), while regarding to primary HHV-6 infection. HHV-6 was detected via PCR technique, and appear that 21 out of 105 patients for febrile convulsions was regarding of the virus [28] and found that, 17% (26/156) of these children had febrile convulsion with either HHV-6, the infection. Children who progress seizures in response to these common viruses might harbor genes that cause them to be susceptible. The virus often data in the improvement of extra deferent for the convulsions, like prolonged seizures, partial seizures, and repeated seizures. It may be a risk parameter for subsequent improvement of epilepsy [27]. The considerable difference in the incidence of HHV-6 infection in children might be related to demographic characteristic of the studied groups such as age of patients or different sensitivity and specificity of the particular assays. Clinical characteristics of primary infection with HHV6 has been well described and consist primarily of a febrile illness in infants, with seizure as the key complication. It has been reported that persistent HHV6 infection is common in children. In addition, they has been reports of HHV6 DNA in serum and/or cerebrospinal fluid (CSF) or through virus isolation in control individuals. Polymerase chain reaction (PCR) tests for virus in blood was frequently positive in persons with past infection, re-infection with new strains or latency with or without repeated reactivation of human herpes virus 6 (HHV6) [28].

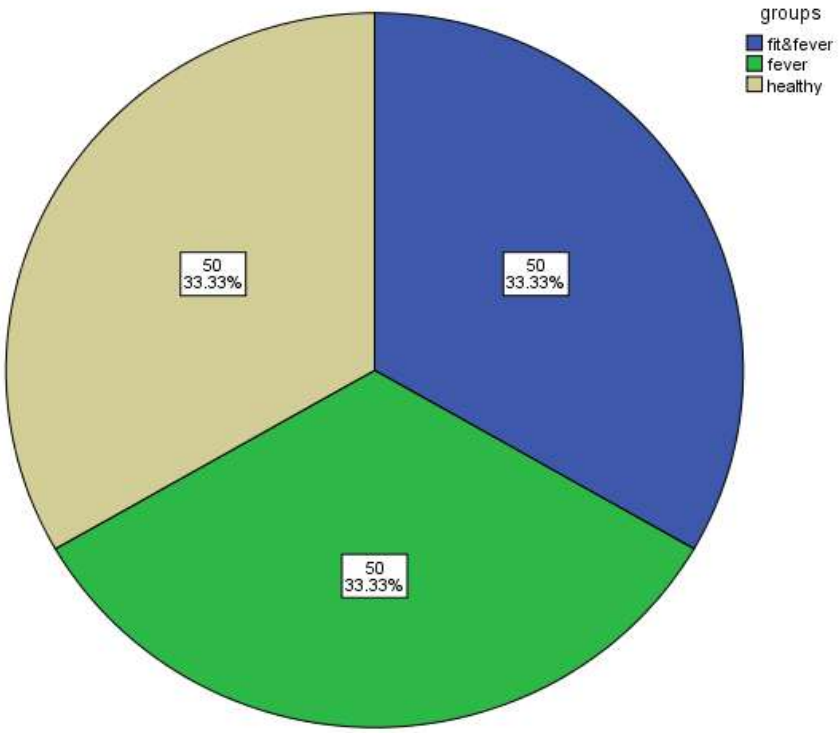

Figure 1: Frequency distribution of the studied groups

DOI: 10.37200/IJPR/V24I5/PR202010
Received: 25 Feb 2020 | Revised: 20 Mar 2020 | Accepted: 30 Mar 2020

Tests based on PCR have assumed an significant part for the laboratory discovery of these agents. Even for HHV6 which can be readily isolated, diagnosis through PCR has become the "gold standard" for some diseases such as febrile convulsion, because infection with different or multiple species of herpes viruses can cause similar symptoms, PCR tests has been designed to detect extra than one herpes virus at a time [29]. A PCR test for herpes viruses for the primers and restriction enzymes initially described through[30]was introduced into our laboratory. As the sequences of the other human herpes virus genomes became available, the PCR was redesigned and a comprehensive test.

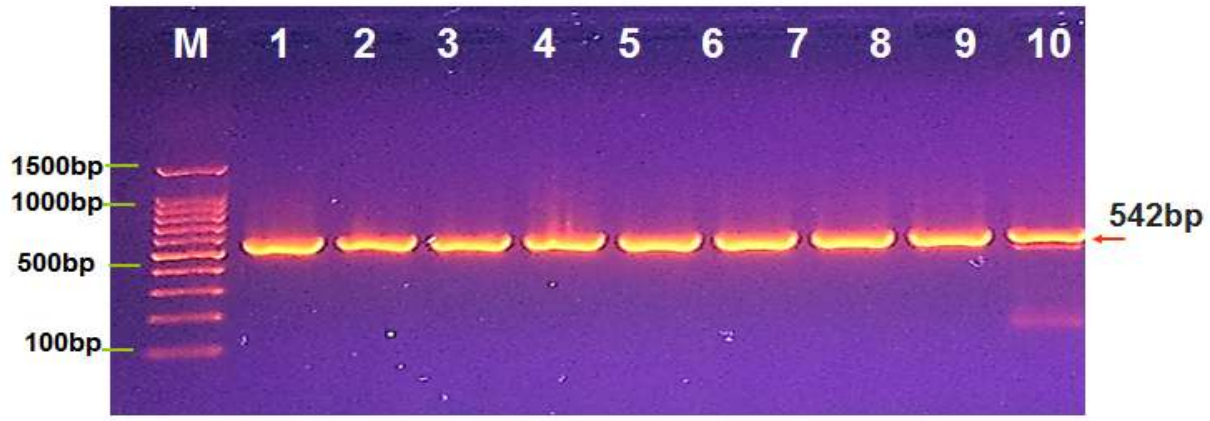

Figure 2: Aga rose gel electrophoresis image that showed PCR product analysis for major capsid protein *MCP* gene in HHV-6 Human patients' blood samples. M (Marker ladder 1500-100bp). Lane (1-10) some positive MCP gene in at 542bp product size

REFERENCES

[1] Razonable, R. R. (2019). Herpes Simplex Viruses 1 and 2, Varicella Zoster Virus, and Human Herpes Viruses 6,7, and 8 in Transplant Recipients. *In Principles and Practice of Transplant Infectious Diseases* (pp. 667-677). Springer, New York, NY.

[2] Pellett, P. E., Ablashi, D. V., Ambros, P. F., Agut, H., Caserta, M. T., Descamps, V. Kuehl, U. (2012). Chromosomally integrated human herpesvirus 6: questions and answers. *Reviews in medical virology*, 22(3), 144-155.

[3] Gantt, S., Orem, J., Krantz, E. M., Morrow, R. A., Selke, S., Huang, M. L. Casper, C. (2016). Prospective characterization of the risk factors for transmission and symptoms of primary human herpesvirus infections among Ugandan infants. *The Journal of infectious diseases*, 214(1), 36-44.

[4] Torii, Y., Kawano, Y., Sato, H., Fujimori, T., Sasaki, K., Kawada, J. I. Ito, Y. (2017). Metabolome analysis reveals the association between the kynurenine pathway and human herpesvirus 6 encephalopathy in immunocompetent children. *Metabolomics*, 13(11), 126.

[5] Ogata, M., Satou, T., Kawano, R., Takakura, S., Goto, K., Ikewaki, J., & Ohtsuka, E. (2010). Correlations of HHV-6 viral load and plasma IL-6 concentration with HHV-6 encephalitis in allogeneic stem cell transplant recipients. *Bone marrow transplantation*, 45(1), 129.

[6] Caviness, K. E. (2015). Complex Gene Expression and Interplay of the UL136 Protein Isoforms Influence Human Cytomegalovirus Persistence.

[7] Engdahl, E. (2016). Human herpesvirus 6A and 6B: assay validation, virus-host interaction and clinical relevance.

[8] Zafar, S., Sorsa, S., Siurala, M., Hemminki, O., Havunen, R., Cervera-Carrascon, V., Kanerva, A. (2018). CD40L coding oncolytic adenovirus allows long-term survival of humanized mice receiving dendritic cell therapy. *OncoImmunology*, 7(10), e1490856.

DOI: 10.37200/IJPR/V24I5/PR202010
Received: 25 Feb 2020 | Revised: 20 Mar 2020 | Accepted: 30 Mar 2020

[9] De Pelsmaeker, S., Romero, N., Vitale, M., & Favoreel, H. W. (2018). Herpesvirus evasion of natural killer cells. *Journal of virology*, 92(11), e02105-17.

[10] Joseph, O., Valéria, K., Károly, N., Barbara, M., Luca, C. N., & Dharam, A. (2011). In Vitro and In Vivo Transactivation of HIV-1 by Human Herpesvirus 6. *HIV-Host Interactions*, 257.

[11] Laina, I., Syriopoulou, V. P., Daikos, G. L., Roma, E. S., Papageorgiou, F., Kakourou, T., & Theodoridou, M. (2010). Febrile seizures and primary human herpesvirus 6 infection. *Pediatric neurology*, 42(1), 28-31.

[12] Gourabi, H. E., Bidabadi, E., Cheraghalipour, F., Aarabi, Y., & Salamat, F. (2012). Febrile seizure: demographic features and causative factors. *Iranian journal of child neurology*, 6(4), 33.

[13] Mahmood, N. S. (2017). Predictive Factors for Complex Versus Simple Febrile Convulsion In Children. *Diyala Journal of Medicine*, 12(2), 87-98.

[14] Sfaihi, L., Maaloul, I., Kmiha, S., Aloulou, H., Chabchoub, I., Kamoun, T., & Hachicha, M. (2012). Febrile seizures: an epidemiological and outcome study of 482 cases. *Child's Nervous System*, 28(10), 1779-1784.

[15] Houshmandi, M. M., Moayedi, A., Rahmati, M. B., Nazemi, A., Fakhrai, D., & Zare, S. (2015). Human Herpes Virus Type 6 and Febrile Convulsion. *Iranian journal of child neurology*, 9(4), 10.

[16] Sharif, M. R., Kheirkhah, D., Madani, M., & Kashani, H. H. (2016). The relationship between iron deficiency and febrile convulsion: a case-control study. *Global journal of health science*, 8(2), 185.

[17] Rabbani, M. W., Ali, I., Latif, H. Z., Basit, A., & Rabbani, M. A. (2013). Serum zinc level in children presenting with febrile seizures. *Pakistan journal of medical sciences*, 29(4), 1008.

[18] Kariuki, S. M., Schubart, C., & Newton, C. R. (2018). The association between human herpes viruses and acute symptomatic seizures in Kenyan children. *Journal of the International Child Neurology Association*, 17-17.

[19] Glauser, T., Shinnar, S., Gloss, D., Alldredge, B., Arya, R., Bainbridge, J. and Jagoda, A. (2016). Evidence-based guideline: treatment of convulsive status epilepticus in children and adults: report of the Guideline Committee of the American Epilepsy Society. *Epilepsy currents*, 16(1), 48-61.

[20] Namakin, K., Zardast, M., SHARIFZADEH, G., BIDAR, T., & ZARGARIAN, S. (2016). SerumTrace elements in febrile seizure: A Case-control study. *Iranian journal of child neurology*, 10(3), 57.

[21] Heydarian, F., Bakhtiari, E., Yousefi, S., & Heidarian, M. (2018). The First Febrile Seizure: An Updated Study for Clinical Risk Factors. *Iranian Journal of Pediatrics*, 28(6).

[22] Dai, Y. J., Xu, Z. H., Feng, B., Xu, C. L., Zhao, H. W., Wu, D. C.,... & Chen, Z. (2014). Gender difference in acquired seizure susceptibility in adult rats after early complex febrile seizures. *Neuroscience bulletin*, 30(6), 913-922.

[23] Zareifar, S., Hosseinzadeh, H. R., & Cohan, N. (2012). Association between iron status and febrile seizures in children. *Seizure*, 21(8), 603-605.

[24] Hill, J. A., & Venna, N. (2014). Human herpesvirus 6 and the nervous system. *In Handbook of clinical neurology* (Vol. 123, pp. 327-355). Elsevier.

[25] Farshadmoghadam, H., Pourakbari, B., Mahmoud, S., Sadeghi, R. H., & Mamishi, S. (2014). Human herpesvirus 6 infection in febrile children: frequency in an Iranian referral hospital. *British journal of biomedical science*, 71(3), 108-110.

[26] Kariuki, S. M., Schubart, C., & Newton, C. R. (2018). The association between human herpes viruses and acute symptomatic seizures in Kenyan children. *Journal of the International Child Neurology Association*, 17-17.

[27] Oikawa, J., Tanaka, J., Yoshikawa, T., Morita, Y., Hishiki, H., Ishiwada, N. and Kohno, Y. (2014). An immune competent child with chromosomally integrated human herpesvirus 6B accidentally identified during the care of Mycoplasma pneumoniae infection. *Journal of Infection and Chemotherapy*, 20(1), 65-67.

[28] Karlsson, T., Mannonen, L., Loginov, R., Lappalainen, M., Höckerstedt, K., & Lautenschlager, I. (2012). Development of a new quantitative real-time HHV-6-PCR and monitoring of HHV-6 DNA aemia after liver transplantation. *Journal of virological methods*, 181(1), 25-36.

[29] Danielson, P. B., McKiernan, H. E., & Legg, K. M. (2017). Integrated Polymerase Chain Reaction Technologies (Sample-to-Answer Technologies). *In Molecular Diagnostics* (pp. 59-78). Academic Press.

[30] Agut, H., Bonnafous, P., & Gautheret-Dejean, A. (2015). Laboratory and clinical aspects of human herpesvirus 6 infections. *Clinical microbiology reviews*, 28(2), 313-335.

DOI: 10.37200/IJPR/V24I5/PR202010
Received: 25 Feb 2020 | Revised: 20 Mar 2020 | Accepted: 30 Mar 2020

View publication stats