ORIGINAL ARTICLE

The Incidence of Human Herpesvirus 6 Infection in Children with Febrile Convulsion admitted to the University Hospital, Kuala Lumpur

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Summary

From October 1996 to March 1997, 31 children with febrile convulsions were admitted to the University Hospital, Kuala Lumpur. Human Herpesvirus 6 (HHV 6) was virologically and/or serologically confirmed to be the cause of the febrile episode in 5 of these children (16.1%). Age, sex and other associated clinical features (diarrhoea, cough, running nose and type of seizure) were not useful in differentiating cases of febrile convulsion due to HHV 6 from those of other aetiology. However, uvulo-palatoglossal junctional ulcers were noted in children in whom the cause of the seizure could be attributed to HHV 6 but not in the remaining cases in the study group.

HHV 6 DNA was detected in peripheral blood mononuclear cells from all patients with febrile convulsions attributed to HHV6, and in patients shown serologically to have already been exposed to the virus by nested polymerase chain reaction amplification. Only genotype HHV 6B was detected from patients with seizure due to HHV 6 but both genotype 6A and 6B were detected in the remaining cases studied.

Key Words: Human Herpesvirus 6, Febrile convulsion

Introduction

Febrile convulsion is a common childhood illness and accounts for a substantial number of paediatric admissions to hospital[1]. The infective agents responsible for this condition are multiple. Until recently, the causative agents for the febrile episodes were not known in a proportion of children with febrile convulsion and these were suspected to be of viral origin. In 1988, Yamanishi *et al*[2] isolated Human Herpesvirus 6 (HHV 6) from 4 infants with exanthem subitum (ES) and all these 4 infants showed seroconversion to the virus. Several other studies on ES have shown that febrile convulsion was an important complication of ES[3,4,5]. Of late, there has been renewed interest in HHV 6 as the possible causative agent of febrile convulsion in children[6] and its possible neurological complications and sequalae[7,8,9,10,11]. This study was undertaken to determine the proportion of children with febrile convulsion admitted to the University Hospital, Kuala Lumpur that could be attributed to HHV 6 infection and whether there are specific clinical features that may help to differentiate seizures due to HHV 6 from those due to other causes.

Materials and Methods

All children with a clinically confirmed febrile convulsion between October 1996 and March 1997

admitted to the University Hospital, Kuala Lumpur were included in this study. Those children with meningitis, encephalitis, past history of epilepsy, suspected poisoning or reaction to vaccine were excluded. Simple febrile convulsion is defined as a seizure lasting less than 15 minutes, with not more than one episode within 24 hours and not followed by post-seizure focal neurological signs, while complex febrile convulsion has one or more of the stated features. Lumbar puncture was not routinely performed in all cases and was only done with parental consent and at the discretion of the attending paediatrician. During the acute phase of the illness, 1.5 ml of venous blood was collected by venipuncture into EDTA tubes using a sterile 21-gauze needle at the time when the blood was taken for other laboratory investigation. Similarly, 1.5 ml of convalescent venous blood was again collected 10 to 14 days later. All blood samples were transported in ice to the laboratory within 6 hours of collection and separated into plasma (for serological testing) and packed cells by centrifugation at 800X g for 10 minutes. The packed cells were resuspended with 5 ml of phosphate buffer saline (PBS) and was centrifuged in Ficoll-paque density gradient at 600X g for 15 minutes to harvest the peripheral blood mononuclear cells (PBMC).

Viral isolation and detection by immunofluorescence

The harvested PBMC were cultured in tissue culture flasks containing 5 ml of RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), interleukin-2 (IL-2, 5 units/ml) and phytohemagglutinin (PHA, 5 µg/ml). The flasks were incubated at 37°C with 5% carbon dioxide atmosphere. After 24 to 48 hours of incubation, the culture medium was changed to RPMI 1640 containing only 10% FCS. The PBMC were examined daily for any cytopathic effects. After 5 to 7 days of incubation, 0.2 ml of the PBMC culture was harvested and washed twice with PBS before final resuspension in 50 µl of PBS. 10 µl of the resuspended PBMC was spread over each well of the Teflon coated slide, dried over a warm plate and subsequently fixed with cold acetone for 10 minutes. The cells were probed for the presence of HHV-6 specific antigen by indirect immunofluorescence using various monoclonal antibodies [OHV-1 (specific for HHV-6A and HHV-6B), OHV-3 (specific for HHV-6B), KR-4 (specific for HHV-7)]; [Gp110 (specific for HHV-6A) - from Rockland, U.K.] followed by a second antibody conjugated with fluorescein isothiocyanate before being viewed under X40 objective using the BH2-RFCA Olympus UV microscope.

On the same day, 1.5 ml of the PBMC culture was co-cultured with 5 ml (1 to 3 X 10^5 cells per ml) of activated human cord blood mononuclear cells (HCBMC) and 5 ml of activated HCBMC was cultured as a control to ensure that no virus isolate was derived from them. After 3 to 6 days of co-cultivation, the leukocytes were observed for cytopathic effect and harvested for detection of HHV 6 antigens by indirect immunofluorescence.

Detection of viral genome by nested polymerase chain reaction.-

The PBMC were suspended in K-buffer (50 mM KCL, 10 mM Tris-HCL [pH 8.3], 3 mM $MgCl_2$, 0.45% Nonidet P-40, 0.45% Tween 20) containing proteinase K (100 µg/ml) at a concentration of 2 x 10^6 cells per 200 µl. The suspension in a microfuge tube was incubated in a water bath at 60°C for 12 hours and subsequently for 10 minutes at 98°C to inactivate the proteinase K. 10 µl of each sample (containing 10^5 cells) was added to 40 µl of reaction mixture [50 mM KCl, 10 mM Tris-HCl (pH 8.3), 3 mM $MgCl_2$, 200 µM each dNTP and 1.5 units of Taq polymerase Promega)]. The specific oligonucleotide primers were used at 0.3 µM each. The outer and inner primers were to amplifiy the specific region of the putative immediate-early gene locus of HHV 6 and were constructed according to the published sequences by Yamamoto *et al*[12]. The mixture was subjected to 30 amplification cycles of denaturation at 94°C for 1 minute, annealing at 62°C for 1 minute and extension at 72°C for 1 minute. An additional extension step of 10 minutes at 72°C was added at the end of the 30th cycle after which 2 µl of the PCR product was used as the template for the nested PCR. The conditions were similar to the first PCR except that the inner primers were used. The resulting amplified products were electrophoresed in 1% agarose

gel containing ethidium bromide and viewed with a UV transilluminator at 302 nm.

Serology

Determination of HHV 6 specific IgG:

Blood was collected from the infants during acute and convalescent phases of the illness and the plasma samples tested for HHV-6 specific IgG and IgM. 5 µl of the plasma was serially diluted with PBS in a microtiter plate and 20 µl of the diluted plasma discharged into respective wells of the Teflon coated slide containing cord blood lymphocytes infected with reference HHV-6B, Hashimato strain. The slide was incubated in a moist chamber for 30 minutes at 37°C, washed several times with PBS and probed with 20 µl of fluorescein isothiocyanate conjugated goat IgG anti-human IgG for 30 minutes at 37°C in a moist chamber. The same process of washing and drying was again repeated and examined under X40 objective using the Olympus BH2-RFCA UV microscope. The end point titer of the dilution was taken as the last well containing at least five discernible fluorescent cells. Each run included one positive, one negative and a blank (PBS) control.

Determination of HHV 6 specific IgM:

5 µl of the patient's plasma was first treated with 45 µl of anti-human IgG (Gullsorb, U.S.A) and the precipitate removed by centrifuging for 10 minutes at 1000X g. The subsequent process for detection and measuring the titer of HHV-6 specific IgM was similar to that described for the HHV-6 specific IgG except the second antibody used to detect the human IgM was fluorescein isothiocyanate conjugated goat IgG anti-human IgM (Rockland, U.K.).

Statistical tests

Fisher exact test and t-test were used to evaluate the significance of the results obtained in this study. All significant results are based on the value of $p < 0.05$.

Results

Table I shows the clinical profiles of the 31 patients with febrile convulsion. Twenty-four of the subjects had simple seizures and the other 7 complex seizures. The ages of the patients ranged from 6 to 29 months, and 18 were males. Cough and coryza were common presenting features and 6 had diarrhoea. Five were found to have uvulo-palatoglossal junctional (UPJ) ulcers. Of the five patients with UPJ ulcers (Table I), two patients (No. 8 and 16) developed maculopapular rash with subsidence of fever on fourth febrile day and the rash faded within 2 days. Two other patients (No. 6 and 29) did not develop such features on defervescence while the fifth patient (No. 3) self-discharged on the third febrile day and failed to return for review.

Paired blood samples were obtained from 11 of the 31 patients and 3 (No. 6, 16, and 29) showed seroconversion to HHV 6 IgM and IgG (Table II). Of the 31 acute samples processed for virus isolation, four were positive for HHV 6B, including two from serologically confirmed cases (No. 6 and 16). Although patient No. 29 was serologically positive, no virus was isolated from the acute specimen collected 12 hours after the fever had subsided.

The mean age of children with FC attributed to HHV 6 was 9.9 months (range 7 - 14 months, SD = 2.7) while the mean age of children with FC due to other causes was 15 months (range 6 - 29 months, SD = 6.2). However, there was no significant age difference in the occurrence of FC due to HHV 6 or other agents ($t = 1.79$, $p = 0.08$). There were also no statistical differences based on gender ($p = 0.625$) or other associated clinical features such as diarrhoea ($p = 1$), cough ($p = 1$), running nose ($p = 1$) and type of seizure ($p = 0.56$). Uvulo-palatoglossal junctional ulcers were present in all the children with FC due to HHV 6 but none noted in the remaining children ($p < 0.001$). The associated clinical features such as diarrhoea, cough and running nose in both sub-groups were mild and self-limiting.

In this study, it was observed that most of the children with FC due to other causes had HHV 6 specific IgG indicating previous exposure to the virus. HHV 6 specific DNA sequence was detected by nested PCR in the PBMC of these children as well as in those cases with FC due to HHV 6 (Figure 1). Only genotype 6B was detected in the PBMC of febrile children due to HHV 6 (Figure 1, lane 3, 6,

Table I
Clinical profiles of children with febrile convulsion

Patient No.	*Age	Sex	Seizure	Diarrhoea	Cough	Coryza	UPJ ulcers
1	11	M	S				
2	17.5	F	S		+	+	
3	14	F	C		+	+	+
4	18	F	C			+	
5	12	M	S		+	+	
6	10	F	S		+	+	+
7	14	M	C	+	+	+	
8	10.5	F	C		+	+	+
9	29	M	S		+	+	
10	11	F	C		+	+	
11	20	F	S		+	+	
12	7	M	S				
13	25	M	S				
14	8	F	S			+	
15	13	M	S	+			
16	7	M	S				+
17	21	M	S	+	+		
18	10	M	C		+	+	
19	6	F	S				
20	11	F	S		+	+	
21	12	F	S		+	+	
22	27	M	S	+	+	+	
23	14	M	S		+	+	
24	8	M	S			+	
25	22.5	F	S		+	+	
26	16	M	S				
27	19	M	S				
28	11	F	C	+			
29	8	M	S	+			+
30	12	M	S				
31	14	M	S				

** Age in month. M (male), F (female), S (simple seizure), C (complex seizure)*
+ indicates presence of that specific symptoms or signs within the column specified

8, 16, 29) and both genotypes 6A and 6B were detected in the PBMC of children with FC due to other causes.

Discussion

In this study, HHV 6 infection occurred in 16.1% of the children with febrile convulsion admitted to the University Hospital which is slightly lower than the 26.1% reported by Barone *et al*[6]. Age, sex and other associated clinical features (diarrhoea, cough, running nose and type of seizure) were not useful in differentating cases of FC due to HHV 6 from those of other aetiology. However, there is a significant association of UPJ ulcers with HHV 6 infection ($p < 0.001$) in children with FC. Thus, the result from this

Table II
HHV 6 isolation and serological profiles of children with febrile convulsion

Patient No.	HHV 6 isolation	IgM titre Acute	IgM titre Convalescent	IgG titre Acute	IgG titre Convalescent
1	-	1 : 10		1 : 80	
2	-	1 : 20		1 : 160	
3	HHV 6B	1 : 10		<1 : 10	
4	-	1 : 40		1 : 320	
5	-	1 : 10	1 : 10	1 : 320	1 : 320
6	HHV 6B	1 : 10	1 : 80	<1 : 10	1 : 320
7	-	1 : 10	<1 : 10	1 : 320	1 : 320
8	HHV 6B	1 : 10		<1 : 10	
9	-	1 : 10		1 : 640	
10	-	1 : 20		1 : 640	
11	-	1 : 20		1 : 320	
12	-	<1 : 10	<1 : 10	<1 : 10	<1 : 10
13	-	1 : 20	1 : 20	1 : 320	1 : 320
14	-	1 : 20		1 : 640	
15	-	1 : 10		1 : 320	
16	HHV 6B	1 : 10	1 : 40	<1 : 10	1 : 320
17	-	<1 : 10		1 : 320	
18	-	<1 : 10		1 : 320	
19	-	<1 : 10		<1 : 10	
20	-	<1 : 10		<1 : 10	
21	-	<1 : 10		1 : 320	
22	-	1 : 20		1 : 160	
23	-	1 : 10		1 : 160	
24	-	<1 : 10	<1 : 10	<1 : 10	<1 : 10
25	-	1 : 10	1 : 10	1 : 160	1 : 160
26	-	<1 : 10		1 : 640	
27	-	1 : 10	1 : 10	1 : 640	1 : 640
28	-	<1 : 10		1 : 320	
29	-	1 : 10	1 : 80	<1 : 10	1 : 320
30	-	<1 : 10		1 : 40	
31	-	<1 : 10	<1 : 10	1 : 160	

- No HHV 6 isolated. Empty spaces indicate no second blood specimen available

study suggests that the presence of UPJ ulcers could be a useful indication of an HHV-6 associated FC. To the best of our knowledge, the association of UPJ ulcers with an HHV-6 infection has not been documented before. An on-going study to evaluate the significance of UPJ ulcers with respect to HHV-6 infection in patients with exanthem subitum is being carried out.

As for laboratory diagnosis, isolation of the virus from PBMC and/or demonstration of seroconversion are useful in confirming primary HHV 6 infection. Detection of HHV 6 specific DNA in PBMC by PCR is not a good method to diagnose primary HHV 6 infection as demonstrated here.

It was noted in the study that only genotype HHV

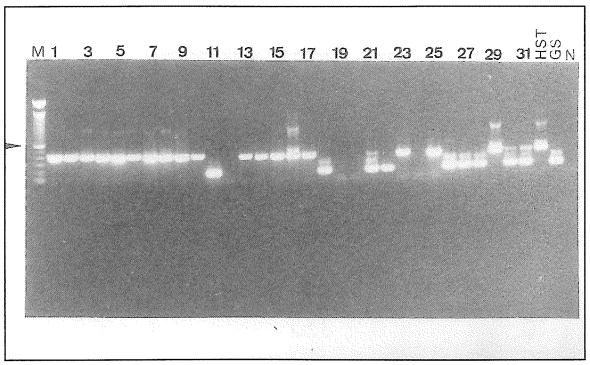

Fig. 1: Ethidium bromide stained agarose gel showing nested PCR products of the acute PBMC of children with febrile convulsion.
The amplified products of the acute peripheral blood mononuclear cells (PBMC) of the respective 31 children with febrile seizure are shown from lane 1 to 31. Human cord blood mononuclear cells infected with HHV 6, Hashimoto strain (lane HST) was used as reference for HHV 6B. Nucleic acid extracted from HSB-2 cells infected with HHV 6, GS strain (lane GS) being used as the reference for HHV 6A. Milli-Q water (lane N) were included as negative control. 100 base-pairs ladder marker (lane M) was used to indicate the molecular size of the amplified products. Arrow head indicates the position of 600 base-pairs.

6B was detected from children with FC attributed to HHV 6 while both genotypes HHV 6A and HHV 6B were detected from children in the sub-group already exposed to the virus. This indicates that both viruses co-circulate in the community and probably genotype B is more virulent in causing symptomatic illness. This finding is in accordance with the report by Dewhurst *et al*[13]. Taking together the results of the nested PCR of acute PBMC and serology, 87.1% of the children had already been exposed to the virus by the age of 3 years old and this is compatible with the previous seroprevalence finding of HHV 6 infection in the local population[14].

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