SEROPREVALENCE OF ANTI-HUMAN HERPESVIRUS-6 IgG ANTIBODY IN CHILDREN OF BANGKOK, THAILAND

Parvapan Bhattarakosol[1], Chitsanu Pancharoen[2], Jutarat Mekmullica[3] and Pattarasinee Bhattarakosol[4]

[1]Department of Microbiology, [2]Department of Pediatrics, Faculty of Medicine, [4]Department of Mathematics, Faculty of Science, Chulalongkorn University, Bangkok 10330; [3]Department of Pediatrics, Bhumibol Adulyadej Hospital, Bangkok 10200, Thailand

Abstract. The prevalence of HHV-6 infection was surveyed by determining the presence of anti-human herpesvirus-6 IgG (Anti-HHV-6 IgG) using an ELISA method. Two hundred and ten sera collected from healthy Thai children aged between 0 to 12 years (mean ± standard deviation = 3.35 ± 3.33) indicated the prevalence of HHV-6 infection was 88.10% (185/210). Samples were classified into 7 groups, 30 samples each, according to their ages, *ie*, group 1: 0 - < 6 months; group 2: 6 - < 12 months; group 3: 12 - <18 months; group 4; 18 - < 24 months; group 5: 2 - < 5 years; group 6: 5 - <8 years and group 7: 8-12 years. The prevalence of HHV-6 infection was 63.33%, 70%, 96.67%, 93.33%, 100%, 100% and 93.33%, respectively. The mean level of anti-HHV-6 IgG among those positive for HHV-6 infection (185 samples) increased from 0 - < 6 months old (17.47 ± 6.32 units) to 27.57 ± 8.42 units in 6 - < 12 months old, with the highest value found in the 18 - < 24 months old group (33.08 ± 8.64 units). The level declined thereafter. A statistically significant difference of the mean level of anti-HHV-6 IgG among positive groups was found (p-value < 0.05). The important factor associated with HHV-6 infection was age (p = 0.002), while sex, socioeconomic status, number of children in the family and child rearing place did not show any association.

INTRODUCTION

Human herpesvirus-6 (HHV-6) was first isolated from acquired immunodeficiency syndrome patients (Salahuddin *et al*, 1986). The virus is an enveloped icosahedral capsid containing linear double stranded DNA (Pellett and Black, 1996). It is now classified as a member of family herpesviridae, subfamily betaherpesvirinae, along with cytomegalovirus (CMV) and human herpesvirus-7 (HHV-7). HHV-6 is a causative agent for exanthem subitum or reseola infantum in children (Yamanishi *et al*, 1988). Patients with primary HHV-6 infection may or may not develop the disease. In cases which do, most display a rash on the skin which commonly occurs during childhood between 6 months to 3 years of age. The patients develop a high fever of 40-41ºC for 5 days and gross lymphadenopathy as well as convulsions can be found. After reduction of the fever, the rash appears (Asano *et al*, 1994). HHV-6 has a latency property, in that infection can be reactivated and recurrence of the symptoms can occur. In adults, the recurrent symptoms may be a mononucleosis-like syndrome, chronic fatigue syndrome, pneumonitis, fulminant hepatitis and encephalitis (Ablashi, 1994; Torre *et al*, 1998). The route of transmission is still unclear; however it is believed to enter via respiratory and oral routes. Since the virus is able to grow in T lymphocytes (DiLuca *et al*,1994), parenteral routes such as blood transfusion, sexual intercourse, organ transplantation and vertical transmission through the placenta, can not be excluded (Gopal *et al*, 1990; Leach *et al*, 1994; Pellett and Black, 1996; Adams *et al*, 1998). In addition. there are some reports revealing the detection of the HHV-6 genome in acute lymphoblastic leukemias (Daibata *et al*, 1998).

Correspondence: Dr Parvapan Bhattarakosol, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. Tel: (66-2) 256-4471, 256-4132; Fax: (66-2) 252-5952 E-mail: parvapan@chula.ac.th

The prevalence of HHV-6 infection around the world has been reported as varying from 20% to >90% (Ranger *et al*, 1991). All of those studies used immunofluorescence assay (IFA) as a method of choice. In this present study, we observed the prevalence of HHV-6 infection in variously aged groups of healthy Thai children by using an enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Study population

210 Healthy Thai children aged between 0 to 12 years old who attended the Well Baby Clinic, Bhumibol Adulyadej Hospital, Bangkok, Thailand were randomly sampled and classified according to age range into 7 groups, *ie*, 0 - <6 months, 6 - <12 months, 12 - <18 months, 18 - <24 months, 2 - <5 years, 5 - <8 years, and 8-12 years. There were 30 cases in each group.

Clinical specimens

Two milliliters of whole blood were collected from each child after the parents signed a consent form. The serum was then kept at -20ºC until use. All information such as age, sex, socioeconomic status, and rearing place of each child was obtained from their parents by interview.

Determination of anti-HHV-6 IgG

All sera were analyzed for the presence of anti-HHV-6 IgG by an ELISA method using an anti-HHV-6 IgG commercial kit (PanBio, Australia). The principal of the test is the indirect ELISA method. The specific antibodies in serum bind to the HHV-6 antigen coated on a plate well. Those complexes were then detected by anti-human IgG conjugated with horseradish peroxidase. The reaction was next observed after adding tetramethylbenzidine hydrogen peroxide (TMB/H_2O_2) as substrate. The result was determined at 450 nm by spectrophotometer. The concentration of antibody (unit) in the serum was calculated according to the manufacturer's description. Serum in which the amount of antibody was less than 10 units was determined to be negative while serum with 10 units or greater was counted as positive.

Data analysis

This study was descriptively designed. Most of the data is presented as mean, range, percentage and frequency of the samples. Comparison of the amount of antibody among each group was done by using Pearson's chi-square or Fisher's exact tests. In groups with a normal distribution, ANOVA was used whereas the Kruskal-Wallis and Mann-Whitney tests were used in groups with an abnormal distribution. Statistically significance was calculated as p-value < 0.05.

RESULTS

All 210 samples were assayed for the presence of anti-HHV-6 IgG antibody. The prevalence of HHV-6 infection was 63.33, 70, 96.67, 93.33, 100, 100, and 93.33% according to the 7 different age groups (Table 1). The mean prevalence of HHV-6 infection among Thai children aged 3.35 ± 3.33 years old was 88.10% (185/210). However, the prevalence of HHV-6 infection in children aged over 6 months to 12 years old was 92.22% (166/180).

Quantitative data of the total amount of HHV-6 IgG antibody from 210 sera were analyzed and classified as positive or negative HHV-6 infection cases (see Materials and Methods). The mean amount of anti-HHV-6 IgG in the positive cases in each group was determined (Table 1). An increase of the level of anti-HHV-6 IgG antibody was observed from group 1 (0 - <6 months): 17.47 ± 6.32 units to group 2 (6 - <12 months): 27.57 ± 8.42 units and the highest level was in group 4 (18 - <24 months): 33.08 ± 8.64 units declining thereafter. Statistically significant differences of the mean amount of anti-HHV-6 IgG antibody among these groups was observed (p-value < 0.05). However, between group 1 and group 7 (p = 0.31), group 2 and 3 (p = 0.08), group 2 and 5 (p = 0.13), group 3 and 4 (p = 0.05), and

Table 1
Comparison of the amount of anti-HHV-6 IgG among different age groups.
N = 30 in each group.

Group	Age range(year) ($\bar{X}$ ± SD)	Sex		Anti-HHV-6 IgG			
		Male (%)	Female (%)	No. Positive (%)	$\bar{X}$ Positive ± SD Unit	No. Negative (%)	$\bar{X}$ Negative ± SD Unit
1	0 - <0.5 (0.12±0.11)	21 (70)	9 (30)	19 (63.33)	17.47±6.32	11 (36.67)	8.07±1.1
2	0.5 - <1 (0.74±0.15)	15 (50)	15 (50)	21 (70)	27.57±8.42	9 (30)	6.12±2.1
3	1 - <1.5 (1.36±0.16)	16 (53.33)	14 (46.67)	29 (96.67)	31.78±8.17	1 (3.33)	8.07
4	1.5 - <2 (1.44±0.15)	20 (66.67)	10 (33.33)	28 (93.33)	33.08±8.64	2 (6.67)	7.195±2.29
5	2 - <5 (3.51±0.85)	17 (56.67)	13 (43.33)	30 (100)	31.16±7.85	0 (0)	-
6	5 - <8 (5.98±0.67)	19 (63.66)	11 (36.67)	30 (100)	27.31±8.6	0 (0)	-
7	8 - 12 (9.99±1.11)	15 (50)	15 (50)	28 (93.33)	18.19±4.62	2 (6.67)	9.09±0.09
Total	0 - 12 (3.35±3.33)	123 (58.57)	87 (41.43)	185 (88.10)	27.15±9.51	25 (11.90)	7.38±1.80

$\bar{X}$± SD : mean ± standard deviation.

Table 2
Factors that effect HHV-6 infection.

	Anti HHV-6 IgG		p-value
	Positive (N = 174)	Negative (N = 22)	
Age (years)			
Mean ± SD	3.74 ± 3.31	1.43 ± 2.95	0.002
Boys : Girls	103 : 71	12 : 10	0.70
Income (Baht)			
Mean ± SD	16,361.49 ± 16,040.48	15,568 ± 13,312.45	0.824
Number of children	2.01 ± 1.04	2.41 ± 1.26	0.096
Place of rearing 1 : 2 : 3 : 4[a]	93 : 9 : 2 : 70	19 : 1 : 0 : 2	UD

[a]1 = home, 2 = babysitter, 3 = nursary, 4 = school, UD = Undetermined

group 4 and 5 (p = 0.38), there was no significant difference.

In this study, risk factors involving HHV-6 infection besides age were included, *ie*, sex, socioeconomic status, number of children in the family and child rearing place. Complete information was collected from 196 cases (Table 2). Our study clearly showed that the only risk factor that played a role in HHV-6 infection was age (p = 0.002).

DISCUSSION

Although many studies of the prevalence of HHV-6 infection have been done, they mainly used IFA. In this present study, an ELISA method, which has been reported to have

sensitivity and specificity equivalent to IFA, (Sloots *et al*, 1996) was used. The samples were collected from healthy children who attended the Well Baby Clinic, Bhumibol Adulyadej Hospital, Bangkok, Thailand therefore the prevalence rate in this study should show an accurate figure for Thai children in the central part of the country. The results revealed the prevalence of HHV-6 infection in various age groups was high when compared to other previous reports from Thailand. One report revealed only 35% of HHV-6 infection to be found in 6 months old and 55% in 12 months old (Balachandra *et al*, 1991) and another showed 44% at 6 months old (Kositanont *et al*, 1995); whereas we here demonstrate 70% in the age group between 6 to <12 months. Our result also indicate that Thai children become infected possibly starting from 6 months of age, since the prevalence rate of infection was found to be 70% in group 2 (6 - <12 months) and increased to 96.67% in group 3 (12 - <18 months) (Table 1). Kositanont *et al* (1995), however, reported the highest rate of infection occurred during 1-2 years of age (84%). The presence of anti-HHV-6 IgG antibody in children aged below 6 months (63.33%) may come from maternal immunity and protect the children from infection.

Quantitative analysis of the amount of anti-HHV-6 IgG antibody in each sample was done and the mean amounts of that antibody in each group were compared. The level of anti-HHV-6 IgG antibody increased according to the increasing age and declined thereafter until the level of antibody was similar to the age between 0 - <6 months (Table 1). This suggests that after infection the level of antibody is maintained at high level until approximately 5 years, then it reduces and is stable thereafter. The difference in the amount of antibody among groups was statistically significant. The significant difference between group 1 and 2 ($p = 0$) confirms the period of infection (over 6 months old). Evaluation of factors involved in HHV-6 infection such as age, sex, socio-economic status, number of children in the family and child rearing place were included. It appears that age is the most important factor for HHV-6 infection ($p = 0.002$, Table 2).

In conclusion, HHV-6 infection in Thai children occurs commonly in the early age of childhood mostly after 6 months old. This finding is similar to the report of Kangro *et al* (1994) who found the prevalence rate of infection to be highest below one year of age (76-92%). After HHV-6 infection up to 20% of infected children, mostly between age 6-12 months, will develop severe disease and require hospitalization (Pruksananhonda *et al*, 1992). Our group has studied the causes of fever in children with first febrile seizures and found that 8.5% are caused by HHV-6 infection (Pancharoen *et al*, 2000). Thus, HHV-6 infection is an important viral pathogen in children, with more than 90% of children aged over 12 years old already infected.

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