PREVALENCES OF HUMAN HERPESVIRUS 6 AND HUMAN HERPESVIRUS 7 IN NORMAL THAI POPULATION

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Abstract. Prevalences of human herpesvirus 6 (HHV-6) and human herpesvirus 7 (HHV-7) DNA were investigated in normal Thai population. Peripheral blood mononuclear cells(PBMC) and saliva were collected from 238 healthy adults in five provinces which might be a representative of each part of the country, and 120 normal children in one province. Prevalences of HHV-6 DNA PBMC were 45.5 - 74.3% in adults and 78.3% in children, and in saliva, very low prevalences were detected; 5.7 - 8.6% in adults and 15.0% in children, respectively. Additionally, all HHV-6 DNA detected in this study were variant B. Comparingly to those of HHV-7 DNA, the prevalences were significantly higher than those of HHV-6, *ie,* 82.9 - 91.4% in PBMC of adults, 85% in PBMC of children, 84.8 - 89.0% in saliva of adults and 92.5% in saliva of children. HHV-6 and HHV-7 isolation from saliva specimens were also performed. No HHV-6 could be isolated from any samples, whereas, in the present study, HHV-7 could be isolated as 90.0% from children and as 20.0 - 54.5% from adults.

INTRODUCTION

HHV-6 and HHV-7 are recently isolated lymphotropic viruses, In 1988, HHV-6 was reported as a causative agent of exanthem subitum (ES) (Yamanishi *et al,* 1998). HHV-6 is divided into two groups as variant A and variant B, on the basis of *in vitro* characteristics, immunoreactivity, and DNA sequences, and ES is mainly caused by HHV-6 variant B (Dewhurst *et al,* 1993;Yamamoto *et al,* 1994). HHV-6 and HHV-7 are highly prevalent in the population. Infection of HHV-6 and HHV-7 is acquired in early childhood: during the first 2 years for HHV-6, and between the ages of 2 and 5 years in case of HHV-7 (Pellett *et al,* 1992; Black *et al* 1993a). After primary infections, generally, HHV-6 and HHV-7 can establish latent infection with non-integrate viral DNA of non-productive form, which can be detected by polymerase chain reaction (PCR). It is believed that the viruses persist in T-lymphocytes and macrophages of peripheral blood and in salivary glands which secrete the infectious viruses (Frenkel *et al,* 1990; Black *et al,* 1993b; Hidaka *et al,* 1993; Kondo *et al,* 1991).

In Thailand, there were some but not so many evidences demonstrating HHV-6 and HHV-7 prevalences in every aspect, and yet, no DNA prevalences of these viruses among normal population have been reported until now. The seroprevalences of HHV-6 and HHV-7 in Thai population at various age-groups have already been described (Balachandra *et al,* 1989; Sutthent *et al,* 1993; Kositanont *et al,* 1995) which in conclusion, of more than 2 years of age, 58 - 83% were the anti-HHV-6 antibody prevalences, while 67 - 82% were of the anti-HHV-7 antibody. Therefore, in this study, the viral DNA prevalences were clarified among normal Thai population to demonstrate the latency of these viruses. Moreover, this Study will be important basic data for considering the viral pathogenesis in various diseases or complications in Thailand, for instances, in patients with dengue hemorrhagic fever (Balachandra *et al,* 1994), or in HIV-infected population (Jayavasu *et al,* 1997). Furthermore, HHV-6 and HHV-7 isolations from saliva were performed in order to confirm the previous evidences that saliva may be a source of transmission.

MATERIALS AND METHODS

Subjects

Adults: Blood and saliva were collected from 238 healthy individuals, male and female, aged 17-58 years old. The adult subjects were obtained from five provinces of Thailand as 100 subjects from Bangkok, and 33-35 subjects from other provinces as Phitsanulok, Chiang Rai, Khon Kaen and Songkhla which are in each part of Thailand as the central,

Table 1
Prevalences of HHV-6 and HHV-7 DNA in PBMC and saliva of normal adults and children.

Specimens	Total No.	HHV-6 DNA (%)		HHV-7 DNA(%)	
		PBMC	Saliva	PBMC	Saliva
Adults					
Bangkok	100	55(55.0)	6(6.0)	85(85.0)	89(89.0)
Phitsanulok	33	15(45.5)	2(6.1)	29(87.9)	28(84.8)
Chiang Rai	35	23(65.7)	2(5.7)	32(91.4)	31(88.6)
Khon Kaen	35	26(74.3)	2(5.7)	29(82.9)	30(85.7)
Songkhla	35	24(68.6)	3(8.6)	29(82.9)	30(85.7)
Children					
Bangkok	120	94(78.3)	18(15.0)	102(85.0)	111(92.5)

Table 2
HHV-6 and HHV-7 isolations from saliva.

Specimens	No. of isolations	No. of virus isolates (%)	
		HHV-6	HHV-7
Adults			
Bangkok	22	0	12(54.5%)
Phitsanulok	10	0	2(20.0%)
Chiang Rrai	10	0	3(30.0%)
Khon Kaen	10	0	3(30.0%)
Songkhla	10	0	3(30.0%)
Children			
Bangkok	20	0	18(90.0%)

lower-northern, uppermost-northern, north-eastern, and southern part, respectively (Table 1).

Children: Since blood from healthy children could not be obtained according to two-time blood drawing requirement, blood and saliva samples were collected from 120 children aged 2-14 years old that came to hospital for the administrations of diarrhea, measles, mumps, asthma, tonsilitis, bronchitis, thalassemia, sinusitis, peptic ulcer, acute gastroenteritis, appendicitis and check up. And they did not show any typical diagnostic symptoms thought to be related to primary HHV-6 or HHV-7 infectious diseases including ES.

Anti-HHV-6 and anti-HHV-7 antibody detection

In children subjects, blood specimens were collected two times and tested the anti-HHV-7 and the anti-HHV-6 antibody titers by indirect immuno fluorescent antibody assay (IFA). To perform this, standard strains of HHV-6 HST (Yamanishi *et al*, 1998), and HHV-7, KHR (Tanaka *et al*, 1994), were propagated in MT4 and SupTl cells, repectively, as the viral antigens to react with the serial diluted test samples. Anti-human IgG-fluorescein isothiocyanate (FITC) conjugate was used as a secondary antibody.

Sample preparation

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood by centrifugation through the ficoll-hypaque solution and counted by using a viable cell count hemocytometer. Saliva was centrifuged at 2,000*g* for 10 minutes to sediment the cells, and remove supernatant. Then 5 x 10^5 cells of PBMC and sedimented cells from saliva samples were lysed with 50 µl of the lysis buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3, 3 mM $MgCl_2$, 0.45% NP40, 0.45% Tween-20 and 200 µg/ml proteinase K) and incubated at 56°C for 3 hours, and heated at 98°C for 10 minutes.

PCR amplification of HHV-6 and HHV-7 DNA

HHV-6 and HHV-7 DNA were detected from PBMC and saliva by nested PCR. Three microliters of PBMC and saliva lysate prepared as described above were used to amplify with specific primers for HHV-6 and HHV-7 DNA, respectively, as described previously (Yalcin *et al*, 1994). Then each amplified product was confirmed by Southern blot hybridization with the oligonucleotide probes specific for HHV-6 or HHV-7 DNA fragments (Yalcin *et al*, 1994).

HHV-6 and HHV-7 isolation from saliva

Three ml of saliva was centrifuged at 2,000*g* for 10 minutes to remove the cells and filtered through sterile 0.45 µm membrane, then inoculated into phytohemagglutinin (PHA)- stimulated cord blood mononuclear cells (CBMC) by centrifugation at 2,000g for 60 minutes, at 20°C and cultivated in RPMJ-1640 with 10% fetal bovine serum, 5 µg/ml PHA,

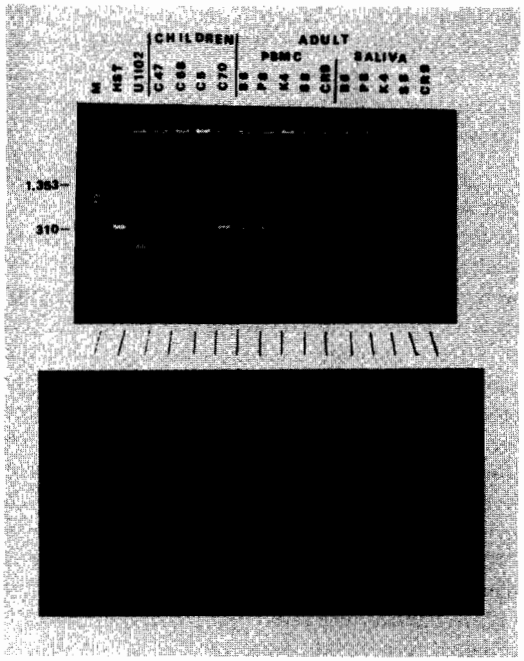

Fig 1–PCR and Southern blot hybridization of HHV-6 DNA in PBMC and saliva of representative Thai normal adults and children run on 2% agarose. HST and Ul 102 were positive controls for HHV-6 variant B and variant A, respectively. C47 and C68, PBMC from children; C5 and C70, saliva from children: B6, P8, K4, S8 and CR9, PBMC and saliva from the individuals taken from Bangkok, Phitsanulok, Khon Kaen, Songkhla and Chiang Rai provinces, respectively. All these samples showed positive results, except the saliva of CR9 was negative of HHV-6 DNA. All positives were of HHV-6 variant B. M, molecular weight markers.

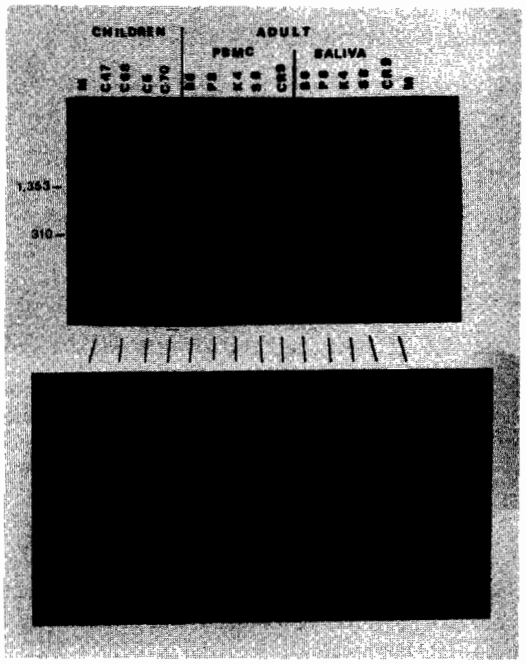

Fig 2–PCR and Southern blot hybridization of HHV-7 DNA in PBMC and saliva of some children and adults run on 2% agarose. C47 and C68 were samples of PBMC whereas C5 and C70 were of saliva from children. Samples of adults were shown as B6, P8, K4, S8, and CR9 for both PBMC and saliva of the same individuals. All these samples showed positive results of HHV-7 DNA. M, molecular weight markers.

and 0.2 unit/ml interleukin-2. The virus isolation were checked by IFA. Briefly, 10^5 cells were collected, mounted on a slide and fixed with cold acetone for 10 minutes, and carried out IFA using 1:100 monoclonal antibody to HHV-6, OHV1 (Okuno *et al*, 1992 a), or 1:100 monoclonal antibody to HHV-7, KR4 (Okuno *et al*, 1992b).

RESULTS

Prevalences of HHV-6 and HHV-7 DNA in PBMC and saliva of normal adults and children

Peripheral blood and saliva were collected from normal adults and children. Adult subjects could be collected from five provinces of Thailand as Bangkok, Phitsanulok, Chiang Rai, Khon Kaen, and Songkhla. All samples of children were obtained only from Bangkok. In each Thai individual, PBMC and saliva were tested for detection of HHV-6 and HHV-7 DNA by PCR amplification followed by Southern-blot hybridization and the representative data were shown in Figs 1 and 2.

Fig 1 showed HHV-6 amplified DNA fragments from PBMC and saliva of both adults and children. Since the virus can be classified into two variants by PCR, the standard strains of variant B (HST), and variant A (U1102) were also amplified to show the sized of each variant. By these amplifications, the fragment of variant B was 432 basepairs in length, whereas that of variant A was 195 basepairs as shown in Fig 1. All PCR products derived from PBMC and saliva of these adults and children tested in this study showed the same size as that of HHV-6 variant B. Fig 2 showed HHV-7 PCR products of PBMC and saliva of representative adults and children, which the amplified fragment was 264 basepairs in length.

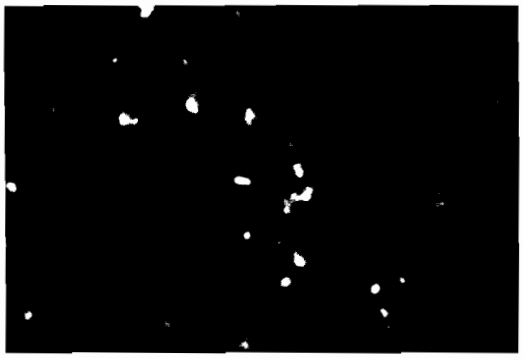

Fig 3–Immunofluorescent assay of HHV-7 isolated from saliva of a Thai children (C 156). Positive signals were observed in saliva inoculated-CBMC after reacting with anti-HHV-7 monoclonal antibody KR4.

The results of HHV-6 and HHV-7 DNA prevalences in PBMC and saliva from adults taken from five provinces of Thailand, and children (only from Bangkok) were summarized in Table 1. HHV-6 DNA prevalences were found in PBMC and saliva of adults as ranging as 45.5 - 74.3% and 5.7 - 8.6%, respectively, and those of children were 78.3% and 15.0%, respectively. In case of HHV-7, the DNA prevalences of adults were 82.9 - 91.4% and 84.8 - 89.0% in PBMC and saliva, respectively, while those of children were 85.0% and 92.5%, respectively.

Virus isolation

HHV-6 and HHV-7 isolations were attempted from the saliva of 62 healthy adults and 20 children who were latently infected with these viruses. The active replication of HHV-6 and HHV-7 were proved by the existences of HHV-6 and HHV-7 antigens by IFA after co-cultivation of the saliva with PHA-stimulated CBMC as described elsewhere. In this study, only HHV-6 but not HHV-7 could be isolated from these saliva, and the HHV-7 infected cells positive by IFA were shown in Fig 3. Table 2 shows the prevalences of HHV-7 isolated from the groups of adult and children saliva. In adults, the prevalences of HHV-7 isolates from the five provinces were ranging as 20.0 - 54.5%, whereas that of children was as 90.0%. In contrast, no HHV-6 could be isolated from the same groups of saliva samples.

DISCUSSION

In this study, specimens of peripheral blood and saliva were taken from individuals, since PBMC is known to be a site for latent infection, and the viral shedding can occur in saliva, moreover, saliva may be a transmission route of infection (Pietroboni *et al*, 1988;Wyatt *et al*, 1992), therefore, it is of interest to compare the prevalences of HHV-6 and HHV-7 between PBMC and saliva.

HHV-6 and HHV-7 DNA prevalences in PBMC and saliva of some normal Thai population were illustrated in this study. Due to HHV-6 DNA, in adults, the prevalences in the five provinces which were in different parts of Thailand were ranging from 45.5 - 74.3% in PBMC and 5.7 - 8.6% in saliva. In PBMC, although the prevalences were not the same in all these five provinces, but were not so significantly different. The most distinctive prevalences were between PBMC and saliva, which very much lower prevalences were found in saliva as compared to those in PBMC. The same events were observed in the prevalences in children, which were 78.3% in PBMC and 15.0% in saliva. Several similar results have been reported by Kido *et al* (1990), Saito *et al* (1991), and Di Luca *et al* (1995) as 3%, 7% and 3% in saliva, respectively. However, higher prevalences of HHV-6 in throat swabs have been reported by Tanaka-Taya *et al* (1996) as 78.6% in 2-8 year-old children and 32.1% in adults. There may be speculated due to not only geographical area difference, but also the sensitivity difference of the test. In the present study, the sensitivity of the PCR for the HHV-6 and HHV-7 detections were the same which followed the method described by Yalcin *et al* (1994) and the sensitivities were lower than those described by Tanaka-Taya *et al* (1996) in the criteria of dNTP, primers, and Taq-polymerase used. Additionally, in that study, HHV-6 PCR had 10 folds higher sensitivity than that of HHV-7.

By PCR followed by Southern-blot hybridization, all specimens of both PBMC and saliva of healthy adults and children could be detected only varitant B in this study. Taken together with other evidences that demonstrating only variant B detected in saliva of healthy adults (Di Luca *et al*, 1994; Aberle *et al*, 1994). From this study, 100% of HHV-6B was found in PBMC of adults and children, However some has reported not fully 100% of variant B in PBMC of healthy young adults but nearly (98%) (Di Luca *et al*, 1994). It is postulated that variant B is more frequently latent than variant A, especially in PBMC. However, in general population, there have been an evidence demonstrating that HHV-6 variant A was predominantly prevalent in cerebrospinal fluid (Hall *et al*, 1998).

According to the HHV-7 DNA prevalences, they were found with high frequency in PBMC and saliva

of both normal adults and children. In adults, the prevalences were not different among these five provinces which were ranging from 82.9 - 91.4% in PBMC and 85.7 - 89.0% in saliva. And these may be representatives to the normal Thai adults. Considerably to those of children, the prevalences were also high and similar to those of adults which were 85.0°/ in PBMC and 92.5% in saliva, respectively. HHV-7 DNA detection was very much more than that of HHV-6 DNA in the same populations. In addition, these findings were in agreement with other reports as 83% in PBMC and 96% in saliva (Kidd *et al*, 1996), and 78.6% and 89.3% in throat swabs of 2-8 year-old children and healthy adults, respectively (Tanaka-Taya *et al*, 1996). From this study, in case of adults from five distinct provinces of Thailand, the prevalences were rather not so different among these five regional areas. Consequently, it is postulated that in each sample, with the same amount of DNA taken, HHV-6 genome was present in lower or more limited amount than HHV-7, especially in saliva.

Attempts were also made to isolate HHV-6 and HHV-7 from saliva of adults and children with the latent infections. Not all saliva specimens but some were taken to isolate the viruses. It is found that no HHV-6 could be isolated from the saliva, and only HHV-7 could be isolated by cocultivation with PHA-stimulated CBMC. Noticeably, as shown in Table 2, the prevalence of replicating HHV-7 in children (90.0%) was very much more than those of adults (20.0 - 54.5%). In case of adults, the highest prevalence was 54.5% which was obtained from specimens in Bangkok which was the nearest site to the laboratory to perform virus isolation, whereas, of the other four provinces were only 20.0 - 30.0%.

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