Malaysian Journal of Medicine and Health Sciences Vol. 8 (2) June 2012: 55-64

Detection of Human Herpesvirus 6 (HHV-6) in Saliva of Healthy Adults in Malaysia

[1]HL Choo, [2]Y Shoji* & [3]CO Leong

[1]School of Postgraduate Studies and Research, International Medical University, School of Pharmacy and Health Sciences, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.
[2]School of Dentistry, International Medical University, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.
[3]School of Pharmacy and Health Sciences, International Medical University, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.

ABSTRACT

Background: Human herpesvirus-6 (HHV-6) levels have been considered as markers for various diseases. The aim of this study was to evaluate the prevalence of HHV-6 infection in healthy adults in Malaysia. **Methods:** The level of HHV-6 in saliva was investigated in 36 healthy adults, age 19 to 23 years, at Kuala Lumpur, Malaysia using variant-specific Taqman™ quantitative real-time PCR (qPCR). **Results:** The amount of HHV-6 DNA in the saliva of healthy adults ranged from negative to 10,000 HHV-6 genomes/ml of saliva (median, 360 genomes/ml of saliva). Of the 36 samples tested, 30 (83%) contained HHV-6 DNA. HHV-6B was the only variant detected in the saliva of all the positive cases. **Conclusions:** The detection of HHV-6 DNA in saliva by real-time PCR assay provides a sensitive and specific quantitation of HHV-6. Our pilot study suggests the wide prevalence of HHV-6 in saliva from healthy adults.

Keywords: Herpesvirus 6, HHV-6, prevalence, real-time PCR, saliva

INTRODUCTION

Human herpesvirus 6 (HHV-6) is an emerging pathogen that was first isolated from patients with lymphoproliferative disorders and acquired immunodeficiency syndrome (AIDS).[1] HHV-6 is classified in the *Betaherpesvirinae* subfamily, along with human cytomegalovirus (HCMV) and HHV-7. There are 2 distinct HHV-6 variants, HHV-6A and HHV-6B.[2] Both variants displayed >90% identical DNA sequence with the exception of a few genes or regions.[3, 4] Despite the high degree of similarity in their DNA content, HHV-6A and HHV-6B have distinct biological properties and association with specific pathological conditions.[5]

HHV-6B has been identified as the cause of exanthem subitum (roseola), a typically mild eruptive childhood disease that is occasionally complicated by development of meningitis, meningoencephalitis, chronic hepatitis or idiophatic thrombocytopenic purpura.[6] When primary infection occurs in teenagers or in adults, the presence of the virus has been associated with hepatitis, encephalitis, mononucleosis syndrome, chronic fatigue syndrome, fatal disseminated infection and, more recently, multiple sclerosis.[6] In contrast, infection with HHV-6A is generally asymptomatic and appears to be unrelated to any specific pathology.[6]

Following primary infection, HHV-6A and HHV-6B are thought to persist for life as a latent form in peripheral blood mononuclear cells (PBMCs), macrophages, and vascular endothelial cells and as a low-level chronic replicating form located in oropharyngeal epithelial cells.[7] A large body of evidence suggests that HHV-6 may act as an opportunistic agent in patients with immunodeficiencies, particularly those who have undergone bone marrow or organ transplantation and human immunodeficiency virus (HIV) infected individuals.[6] A role of HHV-6 as a cofactor in the progression of HIV infection toward full-blown AIDS has also been proposed.[8]

Despite the interest in HHV-6 as an important pathogen, only limited number of population-based studies has evaluated the prevalence of HHV-6 infection in healthy population of developing countries in Asia. This pilot study aimed to investigate the prevalence of HHV-6 and its variant infection in young healthy adults in our population.

MATERIALS AND METHODS

Patients and samples

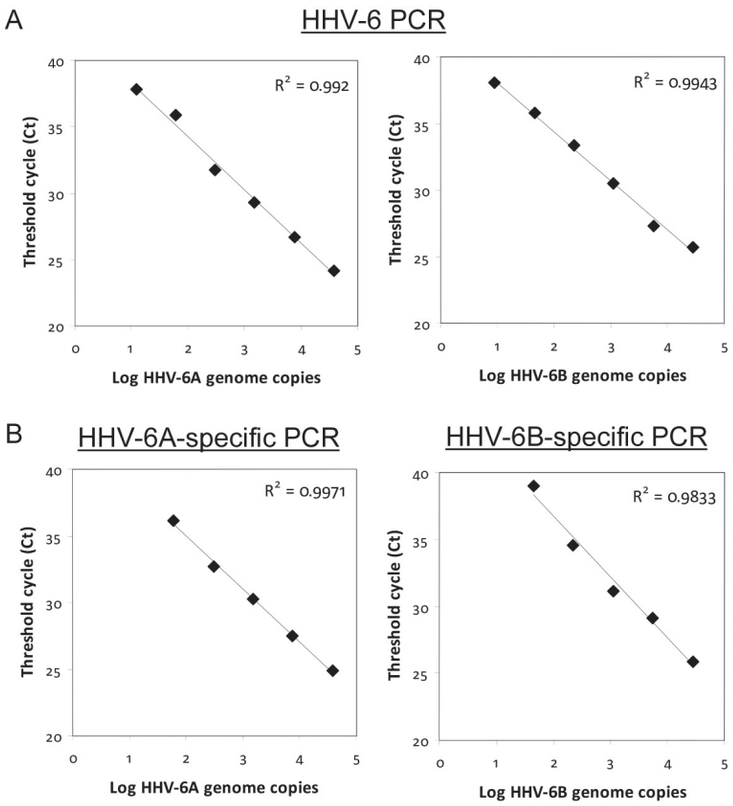
A total of 36 healthy adults (15 males, 21 females) with a median age of 21 years (range, 19 to 23 years) were recruited as volunteers in the present pilot study. Saliva samples (5 ml) were collected through mouth rinses with water. All samples were maintained on ice, divided into 1 ml aliquots and stored at -80°C until use. All subjects were in good

*Corresponding author: *yoshinobu_shoji@imu.edu.my*

general health and did not have histories of liver or kidney dysfunction, symptoms of acute illness (i.e., fever, sore throat, body aches, and diarrhea), or visible oral lesions at the time of enrollment. The study was approved by the International Medical University (Malaysia) Institutional Review Board, and all subjects provided written informed consent as part of the study protocol.

Real-time Quantitative PCR.

DNA in saliva was extracted from 0.5 ml samples using the QIAamp UltraSens Virus kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Real time-PCR was used to detect and quantify HHV-6 in saliva as described previously.[9, 10] Briefly, the 25 µl reaction mixture contained 2 µl of purified DNA from saliva, 12.5 µl TaqMan PCR master mix, 200 nM of each primer, and 100 nM probe specific for HHV-6 (Applied-Biosystem, Carlsbad, CA, USA). The sequence of the forward primer was: 5'-GACAATCACATGCCTGGATAATG-3'. The sequences of the reverse primers specific for HHV-6A, HHV-6B and both variants were: 5'-TGGTAATGGACTAATTGTGTGTTG TTTTA-3'; 5'-TGGTAATGGACTAAGTGT GCGTTATTTTC-3' and 5'-TGTAAGCGTGTGGTAATGGACTAA-3', respectively. The TaqMan® probe was 5'-FAM-AGCAGCTGGCGAAAAGTGCTGTGC-TAMRA-3'. All PCR reactions were performed using a Biorad iQ5 real-time PCR detector system (Bio-Rad, Richmond, CA, USA) and data analyzed using Biorad iQ5 Optical System Software V1.0 as described previously.[11-13] The conditions for all PCR reactions were as follows: 50°C for 2 min, 95 C for 10 min, and 45 cycles of 95°C for 15 s and 60°C for 1 min. Purified DNAs from HHV-6A (GS strain) and HHV-6B (Z29 strain) (Source Bioscience, Nottingham, UK) were used as standard controls. Determination of standard curves for both DNAs was based on triplicate tests over a range of 1 to 104 copies for each reaction. HHV-6 DNA quantitation in saliva was normalized per ml of saliva. The detection limit of the assay were 10 copies of viral genomes per ml of saliva for detection of HHV-6 and 40 copies of genomes per ml of saliva for detection of HHV-6A or HHV-6B without any cross reactivity.



Supplement

Figure 1. Standard curve obtained for HHV-6 quantification. Purified HHV-6A and HHV-6B viral DNA were used to construct the standard curve for (A) overall HHV-6 and (B) variant-specific HHV-6 quantification. Five-fold serial dilutions ranging from 1 copy to 104 copies of DNA were tested in triplicate, and the mean Ct values were plotted against copy number

STATISTICAL ANALYSIS.

The proportions of positive cases obtained for male and female subjects were compared using Fisher's exact test and Mann-Whitney U test. Statistical significance was determined at the $P < 0.05$ level (two-tailed). All data were analyzed with use of the SPSS statistical analysis software V18.

RESULTS

Using the highly sensitive qPCR, we demonstrated that 30/36 (83%) healthy adults were positive for HHV-6 in their saliva. Out of the 36 subjects, 14/15 (93%) male and 16/21 (76%) female were tested positive for HHV-6 DNA. The amounts of HHV-6 DNA per milliliter of saliva vary from 100 to 10,000. Male adults appeared to have higher level of HHV-6 DNA in their saliva (more than 2-fold) compared to female adults. The difference however was not statistically significant.

Table 1. HHV-6 DNA levels in 36 healthy individuals

Subject no.	Age	Gender	HHV-6/ml saliva	HHV-6A	HHV-6B
1	22	Female	-	n.d.	n.d.
2	20	Female	-	n.d.	n.d.
3	21	Female	-	n.d.	n.d.
4	21	Female	-	n.d.	n.d.
5	21	Female	-	n.d.	n.d.
6	21	Female	95	Negative	Positive
7	21	Female	166	Negative	Positive
8	20	Female	173	Negative	Positive
9	21	Female	185	Negative	Positive
10	21	Female	275	Negative	Positive
11	20	Female	311	Negative	Positive
12	20	Female	312	Negative	Positive
13	21	Female	325	Negative	Positive
14	20	Female	396	Negative	Positive
15	20	Female	544	Negative	Positive
16	22	Female	604	Negative	Positive
17	20	Female	749	Negative	Positive
18	20	Female	1,158	Negative	Positive
19	21	Female	1,290	Negative	Positive
20	23	Female	2,769	Negative	Positive
21	21	Female	4,896	Negative	Positive
22	21	Male	-	n.d.	n.d.
23	19	Male	89	Negative	Positive
24	21	Male	96	Negative	Positive
25	22	Male	164	Negative	Positive
26	22	Male	276	Negative	Positive
27	20	Male	490	Negative	Positive
28	22	Male	599	Negative	Positive
29	20	Male	751	Negative	Positive
30	22	Male	984	Negative	Positive
31	23	Male	1,024	Negative	Positive
32	20	Male	1,284	Negative	Positive
33	20	Male	1,781	Negative	Positive
34	20	Male	3,598	Negative	Positive
35	21	Male	4,758	Negative	Positive
36	20	Male	8,208	Negative	Positive

n.d. Not determined

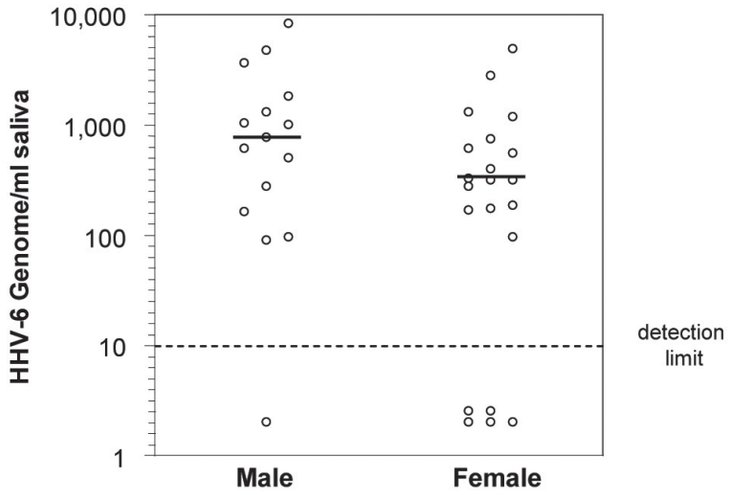


Figure 1. Amount of HHV-6 DNA in saliva of male and female adults. HHV-6 DNA in saliva from healthy adults was determined using real-time PCR. Detection limit was determined to be 10 copies genome/ml of saliva. ○, amount of HHV-6 DNA of each subject; — median value

Table 2. Summary of HHV-6 DNA levels in 36 healthy individuals

	Male	Female	Overall	P Value
HHV-6 positive	14/15 (93%)	16/21 (76%)	30/36 (83%)	0.185[a]
HHV-6 genome/ml saliva				
Mean	1,607 ± 2,273	679 ± 1,158	1,065 ± 1,746	0.163[b]
Median	751	311	360	0.109[c]

[a] Fisher's exact test (2-tailed)
[b] Student's t-test (2-tailed)
[c] Mann-Whitney U test (2-tailed)

To further evaluate the prevalence of different HHV-6 variants in healthy adults, saliva samples that were positive for HHV-6 were further analyzed for HHV-6A and HHV-6B using variant specific qPCR. We found that 30/30 (100%) of the HHV-6 positive healthy adults were positive for HHV-6B in their saliva. The level of HHV-6B detected was consistent with the overall level of HHV-6, suggesting that HHV-6B is the major variant that is presence in the saliva of the healthy adults. This result was further confirmed by the absence of HHV-6A DNA in all the samples being tested. Our results therefore suggest a high prevalence of HHV-6B in saliva of healthy adults in the Malaysian population.

DISCUSSION

The HHV-6 infection is widespread. Its prevalence in healthy adult populations has been reported to range from 0% to 100% depends on the method of detection, sample types, age and geographical locale. A meta-analysis of 4,137 cases from 43 studies reported between 1988 to 2007 reveals that the prevalence of HHV-6 in the sera of healthy individuals was 67.9% (2122/3125) using indirect immunofluorescence assays (IFA) versus 8.8% (16/182) using PCR. Similarly, detection of HHV-6 DNA by PCR in various tissues also yielded a highly variable result (41.1% in PBMCs vs 76.2% in saliva) in healthy populations. It is well accepted that HHV-6 infection usually occurs before 2 years of age and the HHV-6-specific IgG can be detected in almost all newborns, but the prevalence declines to less than 10% by 4 to 5 months of age, increases to 65% by 1 year of age and to greater than 90% by 13 to 36 months of age.[14, 15] Seroprevalence studies in Japan, England, and the United States also demonstrated that infection with HHV-6 is common in the Western world but may differ in population in other geographic/ethnic groups.[16]

Table 3. Meta-analysis of HHV-6 prevalence in healthy individuals

Source	Methods	HHV-6 prevalence, n (%)		Population	Ref
Serum	Immunoassay[1]	37/460	8%	Austria	[32]
		15-Aug	53%	-	[33]
		16-Oct	63%	-	[34]
		29/45	64%	Slovakia	[35]
		33/49	67%	Melanesia	[35]
		297/430	69%	China	[36]
		243/333	73%	Thailand	[37]
		332/434	76%	Brazil	[38]
		502/600	84%	Malaysia	[30]
		153/180	85%	Sweden	[39]
		185/210	88%	Thailand	[40]
		41/43	95%	-	[41]
		59/60	98%	Hungary	[42]
		187/234 HHV-6A	58-80% HHV-6A	Malaysia	[31]
		178/234 HHV-6B	49-76% HHV-6B		
Serum	PCR[2]	0/49	0%	-	[43]
		Jan-46	2%	-	[44]
		Jan-38	3%	-	[45]
		20-Jan	5%	Kuwait	[46]
		29-Dec	41%	-	[47]
PBMC[3]	PCR	29-Jan	3%	-	[48]
		29-Jan	3%	-	[49]
		May-67	7%	-	[50]
		12/150	8%	Latvia	[51]
		Apr-44	9%	-	[52]
		17-Apr	24%	-	[53]
		Dec-42	29%	-	[54]
		14/46	30%	-	[44]
		25-Sep	36%	-	[55]
		16-Jun	38%	Turkey	[56]
		23-Oct	43%	-	[57]
		176/238	74%	Thailand	[58]
		18/20	90%	-	[59]
		18/20	90%	-	[21]
		43/44	98%	-	[22]
Saliva	PCR	19/28	68%	-	[60]
		19/28	68%	-	[61]
		28/38	74%	-	[62]
		26/34	76%	-	[63]
		18/20	90%	-	[21]
		18/20	90%	-	[29]
Tear	PCR	20-Jan	5%	-	[64]
Skin	PCR	13-Mar	23%	-	[65]
Tonsils	PCR	69/69	100%	-	[66]

[1] IFA, indirect immunofluorescence assays (IFA)
[2] PCR includes qPCR, nested-PCR and competitive PCR
[3] PBMC, peripheral blood mononuclear cells

Although HHV-6 antibody titers have been widely used in early clinical studies to identify subgroups of patients with active HHV-6 infection on the assumption that anti-HHV-6 immunoglobulin G (IgG) or IgM titers correlated with viral activity [17-21], a number of recent reports suggests the contrary.[14, 22, 23]. While IgM antibodies have been used to confirm a case of HHV-6 associated roseola or febrile seizures during primary infection, which typically occurs before the age of two, the HHV-6 IgM test is not very useful for adults because it is not produced during viral reactivation.[14] Similarly, elevated IgG antibodies to HHV-6 as an indicator for active, chronic infection also raises controversy as individuals vary in the way they respond to the virus.[14]

Since the majority of healthy individuals have detectable levels of latent virus in their white blood cells, PCR DNA tests of whole blood are not useful unless the test is quantitative, and the absolute level of virus can be compared to a healthy population. When the virus is found in the serum or plasma it is considered a sign of active infection. However, unlike most viral infections where large number of virions spill into the plasma when the virus is replicating, HHV-6 is spread largely from cell-to-cell or directly through the cells walls.[24, 25] Hence, very little free virus spilled in the serum, even in an active infection.

Accumulating evidence suggests that HHV-6 may establish a life-long latent and/or persistent infection in the salivary glands and shedding in saliva of normal healthy individuals.[26-29] Indeed, HHV-6 DNA has been detected in saliva of healthy and HIV positive individuals by PCR with a much higher sensitivity and specificity than IFA or ELISA.[21]

To date, only 2 studies have reported the seroprevalence of HHV-6 in Malaysia, ranging from 49-84% in healthy population.[30, 31] Both studies analyzed sera obtained from a broad-range of age group using IFA. Since HHV-6 has been reported to persist in salivary secretions,[15] we are interested to evaluate the level of HHV-6 DNA in saliva of healthy adult using a highly sensitive and variant-specific qPCR. Our results showed that 83% of the healthy adults were infected with HHV-6, consistent with the previous study reported by Chua *et al.*[30] The levels of HHV-6 DNA range from 100 to 10,000 genome copies per milliliter of saliva.

Further analysis using variant specific qPCR revealed that HHV-6B was the only variant detected in the saliva of the healthy individuals while HHV-6A was not found in the saliva. This result is in contrast to a report by Yadav *et al*. which showed at least 58% of Malaysia population is positive for HHV-6A.[31] The main reason for the discrepancy could be due to the differences in method of analysis and sample types. While we analyzed HHV-6 prevalence using saliva and qPCR, Yadav *et al.* were using serum and IFA. [31] Therefore, we do not rule out the possibility that HHV-6A might be present in other tissues other than oral mucosa or saliva as it has been reported to have a greater tropism in PBMCs or neurons.[6] Regardless, our study suggests the wide prevalence of HHV-6, particularly HHV-6B, among healthy population and suggests that saliva could be the main route of HHV-6 transmission.

CONCLUSION

Our pilot study shows that 83% (30/36) of the Malaysian healthy adults recruited in this small cohort study is infected with HHV-6. Although asymptomatic in healthy adults, the reactivation of the virus has been reported to cause life threatening diseases in immunocompromised individuals such as organ transplant or stem cell recipients and HIV patients. Although there is no drug approved specifically for treatment of HHV-6 infection, drugs used to treat closely related cytomegalovirus (CMV), such as ganciclovir (Cytovene), foscarnet (Foscavir) or cidofovir (Vistide), are widely used in patients with severe HHV-6 reactivation.

In conclusion, we demonstrated that the detection of HHV-6 DNA in saliva by qPCR provides a noninvasive, rapid, sensitive and specific quantitation of HHV-6 in biological samples. This technique will aid to the monitoring of HHV-6 transmission and reactivation in high risk individuals (i.e. immunocompromised patients) which can lead to serious complications. Our study also suggests the wide prevalence of HHV-6 in healthy individuals in Malaysia. Further work is being planned for the detection and quantitation of HHV-6 in diseased individuals using the baseline levels established in this pilot study.

ACKNOWLEDGEMENT

The authors would like to acknowledge Vincent Han Hing Lee, Zeng Zhou Tan and Noor Azura Hani Abdul Razak (School of Dentistry, International Medical Univiersity, Malaysia) for samples collection. The study was supported by the grant IMU 209/2010, Malaysia.

REFERENCES

[1] Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, Halligan G, Biberfeld P, Wong-Staal F, Kramarsky B *et al*. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science 1986; 234: 596-601.

[2] Aubin JT, Collandre H, Candotti D, Ingrand D, Rouzioux C, Burgard M, Richard S, Huraux JM, Agut H. Several

groups among human herpesvirus 6 strains can be distinguished by Southern blotting and polymerase chain reaction. J Clin Microbiol 1992; 30: 2524.

[3] Isegawa Y, Mukai T, Nakano K, Kagawa M, Chen J, Mori Y, Sunagawa T, Kawanishi K, Sashihara J, Hata A *et al.* Comparison of the complete DNA sequences of human herpesvirus 6 variants A and B. J Virol 1999; 73: 8053-8063.

[4] Dominguez G, Dambaugh TR, Stamey FR, Dewhurst S, Inoue N, Pellett PE. Human herpesvirus 6B genome sequence: Coding content and comparison with human herpesvirus 6A. J Virol 1999; 73: 8040-8052.

[5] Drobyski WR, Knox KK, Majewski D, Carrigan DR. Brief report: Fatal encephalitis due to variant B human herpesvirus-6 infection in a bone marrow-transplant recipient. N Engl J Med 1994; 330: 1356-1360.

[6] Agut H. Deciphering the clinical impact of acute human herpesvirus 6 (HHV-6) infections. J Clin Virol 2011; 52: 164-171.

[7] Gopal MR, Thomson BJ, Fox J, Tedder RS, Honess RW. Detection by PCR of HHV-6 and EBV DNA in blood and oropharynx of healthy adults and HIV-seropositives. Lancet 1990; 335: 1598-1599.

[8] Lusso P, Ensoli B, Markham PD, Ablashi DV, Salahuddin SZ, Tschachler E, Wong-Staal F, Gallo RC. Productive dual infection of human CD4+ T lymphocytes by HIV-1 and HHV-6. Nature 1989; 337: 370-373.

[9] Boutolleau D, Duros C, Bonnafous P, Caiola D, Karras A, Castro ND, Ouachee M, Narcy P, Gueudin M, Agut H *et al*. Identification of human herpesvirus 6 variants A and B by primer-specific real-time PCR may help to revisit their respective role in pathology. J Clin Virol 2006; 35: 257-263.

[10] Flamand L, Gravel A, Boutolleau D, Alvarez-Lafuente R, Jacobson S, Malnati MS, Kohn D, Tang YW, Yoshikawa T, Ablashi D. Multicenter comparison of PCR assays for detection of human herpesvirus 6 DNA in serum. J Clin Microbiol 2008; 46: 2700-2706.

[11] Low SY, Tan BS, Choo HL, Tiong KH, Khoo AS, Leong CO. Suppression of BCL-2 synergizes cisplatin sensitivity in nasopharyngeal carcinoma cells. Cancer Lett 2012; 314: 166-175.

[12] Tan BS, Tiong KH, Muruhadas A, Randhawa N, Choo HL, Bradshaw TD, Stevens MF, Leong CO. CYP2S1 and CYP2W1 Mediate 2-(3,4-Dimethoxyphenyl)-5-Fluorobenzothiazole (GW-610, NSC 721648) Sensitivity in Breast and Colorectal Cancer Cells. Mol Cancer Ther 2011; 10: 1982-1992.

[13] Wong SW, Tiong KH, Kong WY, Yue YC, Chua CH, Lim JY, Lee CY, Quah SI, Fow C, Chung C *et al*. Rapamycin synergizes cisplatin sensitivity in basal-like breast cancer cells through up-regulation of p73. Breast Cancer Res Treat 2011; 128: 301-313.

[14] Zerr DM, Meier AS, Selke SS, Frenkel LM, Huang ML, Wald A, Rhoads MP, Nguy L, Bornemann R, Morrow RA *et al*. A population-based study of primary human herpesvirus 6 infection. N Engl J Med 2005; 352: 768-776.

[15] Levy JA, Ferro F, Greenspan D, Lennette ET. Frequent isolation of HHV-6 from saliva and high seroprevalence of the virus in the population. Lancet 1990; 335: 1047-1050.

[16] Levine PH, Neequaye J, Yadav M, Connelly R. Geographic/ethnic differences in human herpesvirus-6 antibody patterns. Microbiol Immunol 1992; 36: 169-172.

[17] Ablashi DV, Salahuddin SZ, Josephs SF, Balachandran N, Krueger GR, Gallo RC. Human herpesvirus-6 (HHV-6) (short review). In Vivo 1991; 5:193-199.

[18] Hoffmann A, Kirn E, Kuerten A, Sander C, Krueger GR, Ablashi DV. Active human herpesvirus-6 (HHV-6) infection associated with Kikuchi-Fujimoto disease and systemic lupus erythematosus (SLE). In Vivo 1991; 5:265-269.

[19] Irving WL, Cunningham AL. Serological diagnosis of infection with human herpesvirus type 6. BMJ 1990; 300: 156-159.

[20] Steeper TA, Horwitz CA, Ablashi DV, Salahuddin SZ, Saxinger C, Saltzman R, Schwartz B. The spectrum of clinical and laboratory findings resulting from human herpesvirus-6 (HHV-6) in patients with mononucleosis-like illnesses not resulting from Epstein-Barr virus or cytomegalovirus. Am J Clin Pathol 1990; 93: 776-783.

[21] Cone RW, Huang ML, Ashley R, Corey L. Human herpesvirus 6 DNA in peripheral blood cells and saliva from immunocompetent individuals. J Clin Microbiol 1993; 31: 1262-1267.

[22] Aberle SW, Mandl CW, Kunz C, Popow-Kraupp T. Presence of human herpesvirus 6 variants A and B in saliva and peripheral blood mononuclear cells of healthy adults. J Clin Microbiol 1996; 34: 3223-3225.

[23] Soldan SS, Berti R, Salem N, Secchiero P, Flamand L, Calabresi PA, Brennan MB, Maloni HW, McFarland HF, Lin HC *et al.* Association of human herpes virus 6 (HHV-6) with multiple sclerosis: Increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA. Nat Med 1997; 3: 1394-1397.

[24] Gautheret-Dejean A, Manichanh C, Thien-Ah-Koon F, Fillet AM, Mangeney N, Vidaud M, Dhedin N, Vernant JP, Agut H. Development of a real-time polymerase chain reaction assay for the diagnosis of human herpesvirus-6 infection and application to bone marrow transplant patients. J Virol Methods 2002; 100: 27-35.

[25] Zhen Z, Bradel-Tretheway B, Sumagin S, Bidlack JM, Dewhurst S. The human herpesvirus 6 G protein-coupled receptor homolog U51 positively regulates virus replication and enhances cell-cell fusion in vitro. J Virol 2005; 79: 11914-11924.

[26] Di Luca D, Mirandola P, Ravaioli T, Dolcetti R, Frigatti A, Bovenzi P, Sighinolfi L, Monini P, Cassai E. Human herpesviruses 6 and 7 in salivary glands and shedding in saliva of healthy and human immunodeficiency virus positive individuals. J Med Virol 1995; 45: 462-468.

[27] Fox JD, Briggs M, Ward PA, Tedder RS. Human herpesvirus 6 in salivary glands. Lancet 1990; 336: 590-593.

[28] Sada E, Yasukawa M, Ito C, Takeda A, Shiosaka T, Tanioka H, Fujita S. Detection of human herpesvirus 6 and human herpesvirus 7 in the submandibular gland, parotid gland, and lip salivary gland by PCR. J Clin Microbiol 1996; 34: 2320-2321.

[29] Jarrett RF, Clark DA, Josephs SF, Onions DE. Detection of human herpesvirus-6 DNA in peripheral blood and saliva. J Med Virol 1990; 32: 73-76.

[30] Chua KB, Khairullah NS, Hooi PS. Seroepidemiology of human herpesvirus 6 in a population seen in the University Hospital, Kuala Lumpur, Malaysia. Southeast Asian J Trop Med Public Health 1996; 27: 91-95.

[31] Yadav M, Umamaheswari S, Ablashi D. Antibody reactivity with two strains of human herpesvirus-6 in Malaysians. J Med Virol 1991; 33: 236-239.

[32] Larcher C, Huemer HP, Honlinger M, Margreiter R, Dierich MP. [Human herpesvirus 6 (HHV-6): Incidence in healthy blood donors and in patients with acute infections caused by other herpes viruses]. Wien Klin Wochenschr 1989; 101: 448-450.

[33] Ghodratnama F, Wray D, Bagg J. Detection of serum antibodies against cytomegalovirus, varicella zoster virus and human herpesvirus 6 in patients with recurrent aphthous stomatitis. J Oral Pathol Med 1999; 28: 12-15.

[34] Okano M, Luka J, Thiele GM, Sakiyama Y, Matsumoto S, Purtilo DT. Human herpesvirus 6 infection and Kawasaki disease. J Clin Microbiol 1989; 27: 2379-2380.

[35] Rajcani J, Yanagihara R. High serum antibody titers to human herpesvirus-6 in Melanesian population. Acta Virol 1994; 38: 121-123.

[36] Wu Z, Mu G, Wang L. Seroprevalence of human herpesvirus-6 in healthy population in two provinces of north China. Chin Med Sci J 1997; 12: 111-114.

[37] Kositanont U, Wasi C, Ekpatcha N, Poomchart A, Likanonsakul S, Suphanip I, Balachandra K, Yamanishi K. Seroprevalence of human herpesvirus 6 and 7 infections in the Thai population. Asian Pac J Allergy Immunol 1995; 13: 151-157.

[38] Linhares MI, Eizuru Y, Stamford WP, Andrade GP, Carvalho Junior LB, Minamishima Y. Cytomegalovirus, human herpesvirus 6 and human T cell leukemia virus infection in renal transplanted patients in the northeast of Brazil. Braz J Med Biol Res 1993; 26: 735-739.

[39] Linde A, Dahl H, Wahren B, Fridell E, Salahuddin Z, Biberfeld P. IgG antibodies to human herpesvirus-6 in children and adults and in primary Epstein-Barr virus infections and cytomegalovirus infections. J Virol Methods 1988; 21: 117-123.

[40] Bhattarakosol P, Pancharoen C, Mekmullica J. Seroprevalence of anti-human herpes virus-6 IgG antibody in children of Bangkok, Thailand. Southeast Asian J Trop Med Public Health 2001; 32: 143-147.

[41] Carricart SE, Bustos D, Biganzoli P, Nates SE, Pavan JV. Isotype immune response of IgG antibodies at the persistence and reactivation stages of human herpes virus 6 infection. J Clin Virol 2004; 31: 266-269.

[42] Stiller I, Pusztai R, Sombor E, Orosz L, Pal A, Tarodi B. Prevalence and avidity of human herpesvirus-6 specific IgG antibodies in pregnant women in Hungary. Acta Microbiol Immunol Hung 2006; 53: 25-34.

[43] Alvarez-Lafuente R, De las Heras V, Bartolome M, Picazo JJ, Arroyo R. Relapsing-remitting multiple sclerosis and human herpesvirus 6 active infection. Arch Neurol 2004; 61: 1523-1527.

[44] Alvarez-Lafuente R, Martin-Estefania C, de Las Heras V, Castrillo C, Picazo JJ, Varela de Seijas E, Gonzalez RA. Active human herpesvirus 6 infection in patients with multiple sclerosis. Arch Neurol 2002; 59: 929-933.

[45] Broccolo F, Drago F, Paolino S, Cassina G, Gatto F, Fusetti L, Matteoli B, Zaccaria E, Parodi A, Lusso P *et al.* Reactivation of human herpesvirus 6 (HHV-6) infection in patients with connective tissue diseases. J Clin Virol 2009; 46: 43-46.

[46] Al-Shammari S, Nelson RF, Voevodin A. HHV-6 DNAaemia in patients with multiple sclerosis in Kuwait. Acta Neurol Scand 2003; 107: 122-124.

[47] Fujiwara N, Namba H, Ohuchi R, Isomura H, Uno F, Yoshida M, Nii S, Yamada M. Monitoring of human herpesvirus-6 and -7 genomes in saliva samples of healthy adults by competitive quantitative PCR. J Med Virol 2000; 61: 208-213.

[48] Rajcani J, Yanagihara R, Godec MS, Nagle JW, Kudelova M, Asher DM. Low-incidence latent infection with variant B or roseola type human herpesvirus 6 in leukocytes of healthy adults. Arch Virol 1994; 134: 357-368.

[49] Blazquez MV, Madueno JA, Jurado R, Fernandez-Arcas N, Munoz E. Human herpesvirus-6 and the course of human immunodeficiency virus infection. J Acquir Immune Defic Syndr Hum Retrovirol 1995; 9: 389-394.

[50] Osman HK, Peiris JS, Taylor CE, Warwicker P, Jarrett RF, Madeley CR. "Cytomegalovirus disease" in renal allograft recipients: is human herpesvirus 7 a co-factor for disease progression? J Med Virol 1996; 48: 295-301.

[51] Kozireva S, Nemceva G, Danilane I, Pavlova O, Blomberg J, Murovska M. Prevalence of blood-borne viral infections (cytomegalovirus, human herpesvirus-6, human herpesvirus-7, human herpesvirus-8, human T-cell lymphotropic virus-I/II, human retrovirus-5) among blood donors in Latvia. Ann Hematol 2001; 80: 669-673.

[52] Sandhoff T, Kleim JP, Schneweis KE. Latent human herpesvirus-6 DNA is sparsely distributed in peripheral blood lymphocytes of healthy adults and patients with lymphocytic disorders. Med Microbiol Immunol 1991;

180:127-134.

[53] Mayne M, Krishnan J, Metz L, Nath A, Auty A, Sahai BM, Power C. Infrequent detection of human herpesvirus 6 DNA in peripheral blood mononuclear cells from multiple sclerosis patients. Ann Neurol 1998; 44: 391-394.

[54] Dolcetti R, Di Luca D, Carbone A, Mirandola P, De Vita S, Vaccher E, Sighinolfi L, Gloghini A, Tirelli U, Cassai E *et al.* Human herpesvirus 6 in human immunodeficiency virus-infected individuals: Association with early histologic phases of lymphadenopathy syndrome but not with malignant lymphoproliferative disorders. J Med Virol 1996; 48: 344-353.

[55] Clark DA, Ait-Khaled M, Wheeler AC, Kidd IM, McLaughlin JE, Johnson MA, Griffiths PD, Emery VC. Quantification of human herpesvirus 6 in immunocompetent persons and post-mortem tissues from AIDS patients by PCR. J Gen Virol 1996; 77 (Pt 9): 2271-2275.

[56] Yalcin S, Karpuzoglu T, Suleymanlar G, Mutlu G, Mukai T, Yamamoto T, Isegawa Y, Yamanishi K. Human herpesvirus 6 and human herpesvirus 7 infections in renal transplant recipients and healthy adults in Turkey. Arch Virol 1994; 136: 183-190.

[57] Ohyashiki JH, Abe K, Ojima T, Wang P, Zhou CF, Suzuki A, Ohyashiki K, Yamamoto K. Quantification of human herpesvirus 6 in healthy volunteers and patients with lymphoproliferative disorders by PCR-ELISA. Leuk Res 1999; 23: 625-630.

[58] Thawaranantha D, Chimabutra K, Balachandra K, Warachit P, Pantuwatana S, Inagi R, Kurata T, Yamanishi K. Prevalences of human herpesvirus 6 and human herpesvirus 7 in normal Thai population. Southeast Asian J Trop Med Public Health 1999; 30: 259-264.

[59] Cuende JI, Ruiz J, Civeira MP, Prieto J. High prevalence of HHV-6 DNA in peripheral blood mononuclear cells of healthy individuals detected by nested-PCR. J Med Virol 1994; 43: 115-118.

[60] Pereira CM, Gasparetto PF, Correa ME, Costa FF, de Almeida OP, Barjas-Castro ML. Human herpesvirus 6 in oral fluids from healthy individuals. Arch Oral Biol 2004; 49: 1043-1046.

[61] Pereira CM, de Almeida OP, Correa ME, Costa FF, de Souza CA, Barjas-Castro ML. Detection of human herpesvirus 6 in patients with oral chronic graft-vs-host disease following allogeneic progenitor cell transplantation. Oral Dis 2007; 13: 329-334.

[62] Lyall EG, Cubie HA. Human herpesvirus-6 DNA in the saliva of paediatric oncology patients and controls. J Med Virol 1995; 47: 317-322.

[63] Collot S, Petit B, Bordessoule D, Alain S, Touati M, Denis F, Ranger-Rogez S. Real-time PCR for quantification of human herpesvirus 6 DNA from lymph nodes and saliva. J Clin Microbiol 2002; 40: 2445-2451.

[64] Pitkaranta A, Piiparinen H, Mannonen L, Vesaluoma M, Vaheri A. Detection of human herpesvirus 6 and varicella-zoster virus in tear fluid of patients with Bell's palsy by PCR. J Clin Microbiol 2000; 38: 2753-2755.

[65] Kempf W, Kadin ME, Kutzner H, Lord CL, Burg G, Letvin NL, Koralnik IJ. Lymphomatoid papulosis and human herpesviruses--A PCR-based evaluation for the presence of human herpesvirus 6, 7 and 8 related herpesviruses. J Cutan Pathol 2001; 28: 29-33.

[66] Roush KS, Domiati-Saad RK, Margraf LR, Krisher K, Scheuermann RH, Rogers BB, Dawson DB. Prevalence and cellular reservoir of latent human herpesvirus 6 in tonsillar lymphoid tissue. Am J Clin Pathol 2001; 116: 648-654.