Prevalence and gB Genotype Distribution of Human Cytomegalovirus among HIV Sero-Negative and HIV Sero-Positive Orphans in Thailand

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Background: *Human Cytomegalovirus (HCMV) infects humans in all geographic areas. Polymorphisms of glycoprotein B (gB) have been used for genotypic characterization of HCMV. However, information of gB genotyping of HCMV in Thailand is not clearly known especially in children.*
Material and Method: *A cross-sectional study was conducted to assess HCMV infection in 236 HIV seronegative and HIV seropositive children who attended an orphanage in Nonthaburi, Thailand by nested-PCR technique using urine specimens. HCMV gB genotypes were determined by restriction fragment length polymorphism (RFLP), and DNA sequencing technique.*
Results: *Sixty-one percent (144/236) of the samples were HCMV positive, which consisted of 66.1% (37/56) of the HIV seropositive children and 59.4% (107/180) of the HIV sero-negative children. Multivariate analysis showed that children who living in one particular room were independently associated with HCMV infection. Genotypic analysis revealed that the most prevalent genotype in these children was gB1; 85.4% (111/130) followed by gB3; 4.6% (6/130), gB2 and gB4 each at 2.3% (3/130). Mixed gB genotypes were identified in 5.4% (7/130) of the samples.*
Conclusion: *HCMV infection, in particular gB1 genotype was commonly identified among these Thai orphans. Living in one particular room was associated with getting the infection. To prevent the transmission of HCMV infection in this setting, improvement in hygienic behavior of childcare workers should be focused.*

Keywords: *Human Cytomegalovirus, Prevalence, gB genotypes, Children, Orphans, Thailand*

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Human Cytomegalovirus (HCMV) is a member of the β-herpes viruses which infects humans in all geographic locations with a prevalence rate of 50-90%. HCMV is also a major cause of increasing morbidity and mortality rates in HIV seropositive patients. Approximately 10-40% of HIV seropositive patients whose CD4+ cell count less than 100 cells/µl will develop HCMV retinitis[1]. The virulence of HCMV infection depends not only on the host immune system, but also on the strains of HCMV. The variation of HCMV genetics in the functionally important genes may influence the virulence and be associated with the symptoms. The UL55 gene encodes viral glycoprotein B (gB) which is the major envelope glycoprotein that involved in host cell entry and cell-cell transmission[2]. Glycoprotein B is considered as an important target for neutralizing antibodies[3]. Genotypic study based on the highly variable region on restriction endonuclease cleavage sites of gB gene provided four patterns of HCMV genotypes: gB1, gB2, gB3 and gB4[4].

The distribution of gB genotypes in different population groups was widely analyzed in order to determine the molecular epidemiology of HCMV infection on whether the correlation of specific gB genotype with the disease really occurs. It is still an open question whether gB genotypes are related to the development of HCMV-associated diseases or which gB genotype mostly influences the infectivity and distribution in certain groups of patients. Determination of frequencies of different HCMV gB genotypes in immunocompetent and immuno-compromised patients as reported by Italian

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researchers showed that gB2 genotype was mostly discovered in bone marrow recipients and AIDS patients[5]. Studies carried out in the group of HCMV congenital infections in southern Hungary revealed that gB1 genotype was commonly found[6]. The gB1 genotype was also the major genotype found in the intrauterine infected infants born in Iowa and Texas, USA[7].

In order to provide epidemiologic information of HCMV infection in Thailand, we investigated the prevalence of HCMV infection together with the distribution of specific gB genotypes in groups of HIV seropositive children compared with HIV seronegative children who attended an orphanage located in Nonthaburi, central Thailand.

Material and Method

Study population

Urine samples were obtained from 236 children who lived in the orphanage, Nonthaburi, Thailand from January 2007 to March 2008. These children included HIV seronegative and HIV sero-positive children that were 56 cases and 180 cases, respectively. The majority of children who attended this orphanage were less than 5 years old. Personal information with clinical status of each subject obtained by way of confidential communication was recorded and was approved by the Ethical Committee, Royal Thai Army Medical Department. Demographic data including gender, age, residential roomand HIV status were recorded with permission and only the coding number of each individual will be available to the public.

Urine samples

An aliquot of 10 ml-urine sample was collected from each subject and centrifuged at 1,200 g for 10 min at 4°C. The viral DNA was isolated by AxyPrep body fluid viral DNA/RNA mini-prep kit (Axygen® Biosciences, USA) according to the manufacturer's instructions.

Nested-PCR RFLP analysis

The HCMV DNA was amplified by using nested-PCR analysis and the primer sets were designed for specific binding with the region of high sequence variability in the HCMV gB gene as previously described[4,8]. The outer primer sequences for the first round of nested-PCR amplification were: gB1246 (5' CGAAACGTGTCCGTCTT3') and gB1724 (5' GAGT AGCAGCGTCCTGGCGA 3'). The inner primers used for the second round nested-PCR amplification were: gB 1319 (5' TGGAACTGGAACGTTTGGCC 3') and gB 1604 (5' GAAACGCGCGCGGCAATCGG 3'). Total volume of the reaction mixture was 50 µl containing 10 mMTris pH 8.3, 50 mMKCl, 2 mM MgCl2, 200 µM of each dNTPs, 2.5 U of Taq DNA polymerase, 0.4 µM of each primers and 10 µl of DNA extracted was used for DNA template. Amplification was performed in a PTC-200 Peltier thermal cycler (MJ Research, Watertown, Mass). The first round of PCR amplification conditions were performed with denaturation at 95°C for 30s, annealing at 58°C for 30s and extension at 72°C for 60s. The cycle was repeated for 35 cycles. The second round PCR was carried out with reaction mixture identical to those used in the first PCR round except the 2 µl PCR product generated from the first round was used as a DNA template. PCR cycle consisted of denaturation at 95°C for 30s, annealing at 67°C for 30s and extension at 72°C for 60s. The positive control, negative control and white control in this experiment were HCMV, DNA, uninfected DNA and water, respectively. The PCR product was analyzed on 1.7% agarose gel, and stained with ethidiumbromide. The gel was observed under UV illumination. To identify HCMV gB genotypes, the PCR product of HCMV positive samples were subjected to HinfI and RsaI digestion (New England Biolabs, UK). Complete digestion was conducted under the condition of 37°C for 16 hrs. The DNA fragments were analyzed on a 12% polyacrylamide gel electrophoresis. The gB genotypes were identified into 4 groups according to the length of DNA fragments (Table 1).

Table 1. Molecular weights of DNA fragments of the 4 gB genotypes after digesting with HinfI and RsaI

Restriction enzyme	DNA molecular weight of gB genotypes (bp)			
	gB1	gB2	gB3	gB4
HinfI	202, 67, 36	202, 100	202, 97	202, 67, 36
RsaI	239, 66	239, 63	195, 63, 41	195, 66, 44

gB = glycoprotein; bp = base pair

The nucleotide sequences were compared to the published gB genotypes sequences of the four major gB genotypes (Gen Bank accession numbers gB1: M60927, M60929, gB2; M60931, M60932, gB3; M60933, M60934, gB4; M60924, M60926). Alignment of the DNA sequences were performed by using the Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 downloaded from a reliable and free website (http://www.megasoftware.net/). Homology of the sample sequences with those of the Gen Bank's reference was determined by using EMBOSS Pairwise Alignment Algorithms Software (free reliable on http://www.ebi.ac.uk)[9].

Statistical analysis

The association between potential risk factors and HCMV infection was assessed by Chi-square test with a 95% confidence interval. Univariate analysis was performed using SPSS for Windows version 11.5 (SPSS, Chicago, IL). Odds ratios with 95% confidence intervals and *p*-values were calculated to compare outcome among study groups. A *p*-value <0.05 was considered statistically significant. Logistic regression was performed for multivariate analysis to assess the independent association of risk factors and HCMV infection.

Results

Prevalence of HCMV infection by nested-PCR analysis

Demographic data and HCMV infection of the children enrolled in the present study are summarized in Table 2. The overall prevalence of HCMV infection in this group of Thai children was 61.0%. Regarding to HIV status, 66.1% of HIV seropositive children versus 59.4% of HIV seronegative children was HCMV-positive. However, the prevalence of HCMV infection among these two groups was not significantly different ($p = 0.375$). The prevalence of HCMV in male versus female children was also not significantly different ($p = 0.453$). Significant differences were observed in the prevalence of HCMV infections among different age ranges and residential rooms. Children who lived in room No. 2 showed a high prevalence of HCMV infection at 82.1%. The high prevalence of the infection was also detected in children who aged between 37-48 months

Table 2. The characteristics of the enrolled orphans and prevalence of HCMV infection

Characteristic	Total n = 236	No. of HCMV positive (%) n = 144	*p*-value
Gender			
Female	93	54 (58.1)	0.453
Male	143	90 (62.9)	
HIV status			
Seropositive	56	37 (66.1)	0.375
Seronegative	180	107 (59.4)	
Age (months)			
0-12	84	39 (46.4)	0.007
13-24	60	40 (66.7)	
25-36	31	24 (77.4)	
37-48	18	15 (83.3)	
49-60	19	12 (63.2)	
>60	19	10 (52.6)	
Room			
No. 1	56	37 (66.1)	<0.001
No. 2	28	23 (82.1)	
No. 3	18	14 (77.8)	
No. 4	27	19 (70.4)	
No. 5	20	10 (50.0)	
No. 6	17	8 (47.1)	
No. 7	20	9 (45.0)	
No. 8	14	1 (7.1)	
No. 9	13	9 (69.2)	
No.10	19	10 (52.6)	

at 83.3%. Whereas, the children who aged between 0-12 months showed the lowest rate of HCMV infection at 46.4% especially those who lived in room No. 8 which only 7.1% were infected with HCMV.

The results of univariate and multivariate analysis of risk factors associated with HCMV infection as shown in Table 3. Univariate analysis indicated that the children who lived in residential room No. 2 were 3.4 times at greater risk of acquiring an infection with HCMV. However, there was no significant association between HCMV infection with gender, HIV status and age ranges. The multivariate analysis also showed that children from residential room No. 2 were independently associated with HCMV infection and had 2.9 times greater risk of getting the infection than the other rooms (OR: 2.9, 95% CI: 1.1-8.0, $p = 0.038$).

Distribution of HCMV glycoprotein B (gB) genotypes

One hundred and forty-four from 236 (61.0%) urine samples were positive by nested-PCR analysis. However, only 90.3% (130/144) of the positive sampleswerere-amplifiable by nested-PCR and used in the genotypic analysis. The overall distribution of HCMV gB genotypes was depicted in Table 4. It is revealed that gB1 genotype was commonly found in both HIV sero-positive (18/26, 69.2%) and HIV seronegative (93/104, 89.4%) children. Mixed gB genotypes were observed in 5.4% (7/130) of samples consisting of 15.4% (4/26) of the HIV seropositive children and 2.9% (3/104) of the HIV seronegative children as shown in Table 5.

DNA sequencing of gB genotype with the pattern of RsaI undigested DNA

The presence of gB genotypes with the pattern of RsaI undigested DNA was detected in 15 of 130 (11.5%) samples. However, the patterns of DNA fragments obtained from HinfI digestion were similar

Table 3. Univariate and multivariate analysis of risk factors associated with HCMV infection

Characteristics	Prevalence of HCMV infection (%)	Total (%)	Crudeodds ratio (95% CI)*	*p*-value	Adjustedodds ratio+ (95% CI)*	*p*-value
Gender						
Female	54 (58.1%)	93 (39.4%)	1			
Male	90 (62.9%)	143 (60.6%)	0.8 (0.5-1.4)	0.454	-	-
HIV status						
Seronegative	107 (59.4%)	180 (76.3%)				
Seropositive	37 (66.1%)	56 (23.7%)	11.3 (0.7-2.5)	0.375	-	-
Age group (m)						
Others	125 (58.7%)	213 (92.2%)				
37-48	15 (83.3%)	18 (7.8%)	13.5 (1.0-12.5)	0.052	-	-
Room						
Others	117 (57.4%)	204 (87.9%)				
No. 2	23 (82.1%)	28 (12.1%)	13.4 (1.3-9.4)	0.017	12.9 (1.1-8.0)	0.038

* CI = confidence interval

+ Adjusted for aged group, gender and HIV status

Table 4. HCMV gB genotypes distribution in the group of HIV seronegative and HIV seropositiveorphans

gB genotype	HIV seronegative orphan (%)	HIV seropositive orphan (%)	Total (%)
gB 1	93 (89.4)	18 (69.2)	111 (85.4)
gB 2	3 (2.9)	-	3 (2.3)
gB 3	2 (1.9)	4 (15.4)	6 (4.6)
gB 4	3 (2.9)	-	3 (2.3)
Mixed	3 (2.9)	4 (15.4)	7 (5.4)
Total	104 (100)	26 (100)	130 (100)

gB, glycoprotein

Table 5. Characteristics of mixed gB genotypes obtained from 7 urine samples of HIV-infected and HIV-uninfected children

HCMV gB genotypes	Children		Total
	HIV-positive	HIV-negative	
2 genotypes			
gB 1, gB 2	1	0	1
gB 1, gB 3	1	0	1
gB 1, gB 4	0	1	1
gB 3, gB 4	0	1	1
3 genotypes			
gB 1, gB 2, gB 4	0	1	1
4 genotypes			
gB 1, gB 2, gB 3, gB 4	2	0	2
Total	4	3	7

gB = glycoprotein

to those of gB 1 and gB 4 genotypes which contained; 202, 67 and 36 bp of DNA fragments.

As shown in Fig. 1, these samples are likely belonged to HCMV gB 1 genotype except for the substitution of A for G in the recognition site of RsaI (5'-GTAC-3'). The constructed phylogenetic tree indicated that these unusual gB genotype samples were closely related to gB 1 genotype (Fig. 2). The homology of the RsaI undigested DNA and the gB genotypes from GenBank's reference sequences were found to be 96.8% homology with DNA sequences of gB 1 genotype GenBank Accession No. M60927. Only 93.7%, 93.3% and 81.8% homology were obtained when this unusual gB genotype was compared with gB 4 (Genbank Accession No. M60926), gB 2 (Genbank Accession No. M60932) and gB 3 (Genbank Accession No. M60934), respectively.

Discussion

The prevalence of HCMV infection in the group of HIV seropositive patients has been reported by various investigators. In 1996, Chandwani et al determined the prevalence of HCMV infection with HIV-1 co-infection in a group of children from USA. From their results, HCMV was recovered mostly in 31% of HIV seropositive children, followed by 17% and 13% in the group of sero-reverters born to HIV sero-positive-mothers-and HIV sero-negative children, respectively[10]. In 2003, Likitnukul et al, found a significant difference of the prevalence of HCMV infection between HIV sero-positive children and HIV sero-negative children aged 13-36 months but not in younger group[11]. Our result demonstrated that no significant difference of HCMV infection in these two groups of children, i.e. HCMV was present in 66.1% of HIV seropositive children and in 59.4% of HIV seronegative children. Similar to this previous report, there was a significant difference of HCMV infection between HIV seropositive children (10/10, 100%) and HIV sero-negative children (41/58, 71.1%) at the age of 25-60 months ($p = 0.044$).

The present study demonstrated that children aged between 37-48 months presented the highest rate of HCMV infection at 83.3% while those aged more than 60 months, and lower than 12 months, had lower rates of HCMV infection. The children lived separately in 10 different residential rooms. Those who were HIV seropositive lived in a room isolated from those who were HIV seronegative. Residential room No. 2, which consisted of HIV sero-negative children mostly aged between 25-48 months, had the highest rate of HCMV infection. The multivariate analysis of risk factors associated with HCMV infection revealed that children who lived in this particular room had 2.9 times greater risk of getting the infection than those who lived in other rooms. Different risks of HCMV transmission are presumably associated with different behaviors of younger and older aged children in performing good personal hygiene practices and the ability to use properly and clean after using the toilet, especially hand washing, which may reduce the risk of HCMV acquisition.

Environments in limited area of the day-care center and the differences in the abilities to perform good personal hygiene practices of various ages of the children were proposed to be associated with the rate of HCMV transmission[12]. Indeed, a later study by Kashiwagi et al, showed a high rate of HCMV infection

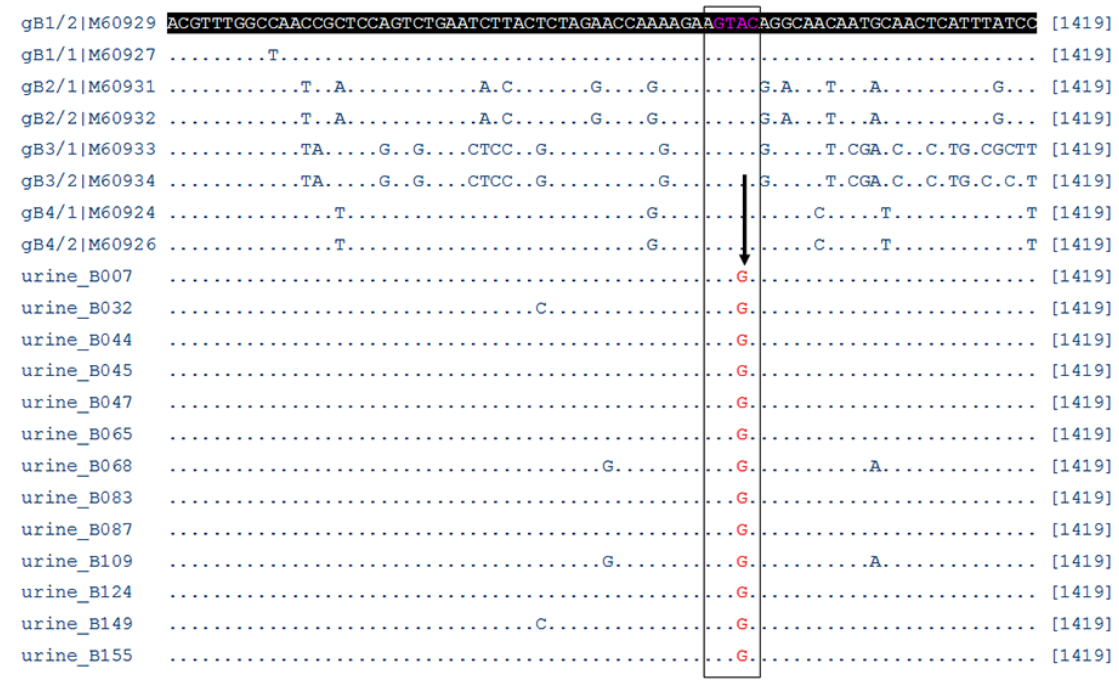

Fig. 1 The DNA sequences alignment of HCMV glycoprotein B which shows a pattern of gB genotype with RsaI undigested DNA. The box shows restriction site of RsaI, "5'-GTAC-3'". The arrow indicates the nucleotide position which differs from that in the database.

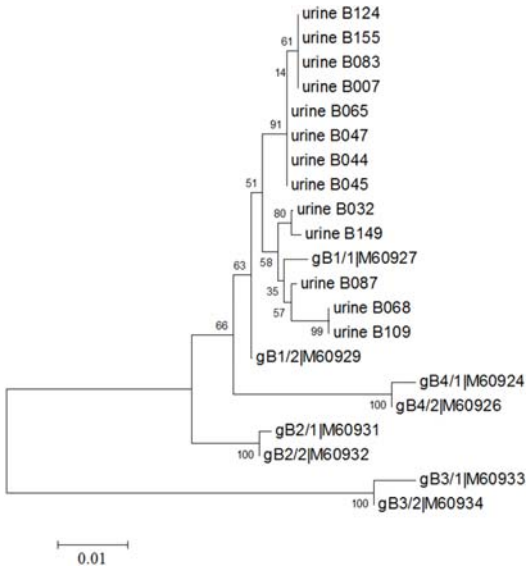

Fig. 2 Phylogenetic analysis of the unusual gB genotypes from samples. The tree was constructed from comparison of gB gene nucleotide sequences by Bootstrap consensus tree analysis.

among children attending aday-care center than those did not[13]. The wide spread of HCMV infection among children in a daycare center might be partly due to HCMV contamination of certain center areas where saliva and urine were excreted from the infected children. The fact that transmission occurred via direct exposure to the urine and saliva by those infected children could be attributed to the habit of placing toys and objects in their mouths due to poor hygienic practices. It probably contributes to the important role of this transmission mechanism among such children who lived in a limited area of a daycare center or a babies' home. The HCMV prevalence among each age range of the children in this study indicates the basic importance of a hygienic environment and good general personal care of the children by their caretakers. Hence, in order to reduce the transmission, the importance of these findings should be taught to those involved including children and caretakers through proper training.

HCMV infection becomes common in many people worldwide. The transmission rate may not only be due to life style and personal hygiene of the individuals, but the HCMV stains may also be involved. The distribution of HCMV gB genotypes among various subsets of the population in different geographic locations has been established and the correlation between gB genotypes with risk factors or pathogenicity has been determined. Studies with those

having retinitis were commonly found with HCMV gB 2 genotype in association with bone marrow transplant recipients and HIV positive patients, whereas the most dominant genotype was found to be gB 1 in association with renal transplant recipients[8,14,15].

Many groups of investigators had studied frequencies of HCMV genotypes found in the group of premature infants and children. In the United States, the prevalence of gB genotypes in association with intrauterine HCMV infections in Iowa and Texas was gB 1 genotype commonly found at 50%, followed by gB 3, gB 2 and gB 4[7]. In addition, this study found gB 1 to be the most common genotype in symptomatic congenitally infected infants[7]. A study with Italian subjects confirmed a possible role for genotype gB 1 in symptomatic congenital HCMV infections at the prevalence rate of 50% in babies[5]. The same result was reported with a group of Japanese children byTanaka et al that gB1 was the most common genotype in congenital HCMV infection. The distribution of gB 1, gB 3 and gB 2 from their study were 64%, 32% and 4%, respectively, while gB 4 genotype was not recognized in their study[16]. The investigation on gB genotypes in congenitally infected infants in China conducted by Yu et al, also indicated that gB 1 was the most prevalent genotype followed by genotypes gB 3 and gB 2, while gB 4 genotype was infrequently found in the present study[17].

For Thailand, studies of genotypic variations among HCMV gB genotypes in clinical samples have been analyzed[18,19], but no report was found with the distribution of HCMV gB genotypes especially in a group of children. Therefore, the present study is the first report of HCMV gB genotypes in a group of Thai children. The dominance of gB 1 genotype in Thai children is similar to those reported in children from other countries as described above. However, the prevalence rate of HCMV gB 1 genotype obtained from this study, i.e. 85.4%, is remarkably higher than those reported in the previous studies.

The present study found unusual gB genotypes patterns as indicated by the RFLP results of the 3 digested DNA fragments for gB 1 and gB 4 (202, 67 and 36 bp). In addition, a pattern of undigested DNA by RsaI digestion was detected as shown with the PCR amplicons sized 299-305 bp on the agarose gel electrophoresis. Alignment of this amplicons with published gB genotype sequences indicated that they most likely belong to HCMV gB 1 with 96.8% homology and with nucleotide "A" (adenine) in the recognition site of RsaI (5'-GTAC-3') was substituted with nucleotide "G" (guanine) (5'- GTGC-3') as shown in Fig. 3. The single nucleotide substitution in the recognition site of restriction enzyme RsaI of HCMV UL55 gene found from the present study has not been previously reported by any group. The result of phylogenetic analysis of the unusual gB clearly confirmed the relationship of these samples to gB1 genotype.

The present study also emphasizes the distribution of HCMV genotypes specific to a certain population and their association with the host status. Limitation of this study was due to the lack of clinical data of the patients. These include clinical observations, long-term follow-up for HCMV determination, level of CD4+ lymphocyte counts, clinical manifestation for the development of AIDS and the drug regimens employed. Therefore, the correlation of gB genotypes and clinical outcomes could not be determined. However, the data generated from the present study provide a basic knowledge of the HCMV genotypes in infection among a group of Thai children who attend the orphanage. This finding may lead to the development of new diagnostic tools and genetic markers for HCMV genotypes in order to identify the infection and indicate the rate of transmission. The finding of the unusual gB1 genotype in this group of children will possibly lead to more studies on mutagenesis analysis of HCMV strains, their importance in the viral-host interaction and also the mechanism of the viral infectivity and virulence.

Conclusion

HCMV infection, in particular gB 1 genotype was commonly identified among the children in this orphanage. Those who lived in one particular room had a higher risk of acquiring the infection. To prevent the transmission of HCMV infection in this setting, improvement in hygienic behavior of childcare workers should be focused.

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Potential conflicts of interest

None.

References

1. Scholz M, Doerr HW, Cinatl J. Human cytomegalovirus retinitis: pathogenicity, immune evasion and persistence. Trends Microbiol 2003; 11: 171-8.
2. Navarro D, Paz P, Tugizov S, Topp K, La Vail J, Pereira L. Glycoprotein B of human cytomegalovirus promotes virion penetration into cells, transmission of infection from cell to cell, and fusion of infected cells. Virology 1993; 197: 143-58.
3. Endresz V, Kari L, Berencsi K, Kari C, Gyulai Z, Jeney C, et al. Induction of human cytomegalovirus (HCMV)-glycoprotein B (gB)-specific neutralizing antibody and phosphoprotein 65 (pp65)-specific cytotoxic T lymphocyte responses by naked DNA immunization. Vaccine 1999; 17: 50-8.
4. Chou SW, Dennison KM. Analysis of interstrain variation in cytomegalovirus glycoprotein B sequences encoding neutralization-related epitopes. J Infect Dis 1991; 163: 1229-34.
5. Arista S, De Grazia S, Giammanco GM, Di Carlo P, Iannitto E. Human cytomegalovirus glycoprotein B genotypes in immunocompetent, immunocompromised, and congenitally infected Italian populations. Arch Virol 2003; 148: 547-54.
6. Lukacsi A, Tarodi B, Endreffy E, Babinszki A, Pal A, Pusztai R. Human cytomegalovirus gB genotype 1 is dominant in congenital infections in South Hungary. J Med Virol 2001; 65: 537-42.
7. Bale JF, Jr., Murph JR, Demmler GJ, Dawson J, Miller JE, Petheram SJ. Intrauterine cytomegalovirus infection and glycoprotein B genotypes. J Infect Dis 2000; 182: 933-6.
8. Carraro E, Granato CF. Single human cytomegalovirus gB genotype shed in multiple sites at the time of diagnosis in renal transplant recipients. J Med Virol 2003; 70: 240-3.
9. Olson SA. EMBOSS opens up sequence analysis. European Molecular Biology Open Software Suite. Brief Bioinform 2002; 3: 87-91.
10. Chandwani S, Kaul A, Bebenroth D, Kim M, John DD, Fidelia A, et al. Cytomegalovirus infection in human immunodeficiency virus type 1-infected children. Pediatr Infect Dis J 1996; 15: 310-4.
11. Likitnukul S, Bhattarakosol P, Poovorawan Y. Seroprevalence of cytomegalovirus infection in children born to HIV-1 infected women. Asian Pac J Allergy Immunol 2003; 21: 127-30.
12. Pass RF, Hutto SC, Reynolds DW, Polhill RB. Increased frequency of cytomegalovirus infection in children in group day care. Pediatrics 1984; 74: 121-6.
13. Kashiwagi Y, Nemoto S, Hisashi, Kawashima, Takekuma K, Matsuno T, et al. Cytomegalovirus DNA among children attending two day-care centers in Tokyo. Pediatr Int 2001; 43: 493-5.
14. Picone O, Costa JM, Leruez-Ville M, Ernault P, Olivi M, Ville Y. Cytomegalovirus (CMV) glycoprotein B genotype and CMV DNA load in the amniotic fluid of infected fetuses. Prenat Diagn 2004; 24: 1001-6.
15. Xanthakos SA, Schleiss MR. Glycoprotein B genotyping of cytomegalovirus strains isolated in a pediatric population. Pediatr Infect Dis J 2003; 22: 462-3.
16. Tanaka K, Numazaki K, Tsutsumi H. Human cytomegalovirus genetic variability in strains isolated from Japanese children during 1983-2003. J Med Virol 2005; 76: 356-60.
17. Yu ZS, Zou CC, Zheng JY, Zhao ZY. Cytomegalovirus gB genotype and clinical features in Chinese infants with congenital infections. Intervirology 2006; 49: 281-5.
18. Bhattarakosol P, Chantaraarphonkun S. Prevalence of human cytomegalovirus (HCMV) gB genotypes in Thai patients. Southeast Asian J Trop Med Public Health 2007; 38: 835-40.
19. Chantaraarphonkun S, Bhattarakosol P. Intra- and intergenotypic variations among human cytomegalovirus gB genotypes. Intervirology 2007; 50: 78-84.

ความชุกและการกระจายของ gB genotype ของ Human cytomegalovirus ในเด็กกำพร้าที่ติดเชื้อและไม่ได้ติดเชื้อเอชไอวีในประเทศไทย

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ภูมิหลัง: *การติดเชื้อ Human cytomegalovirus (HCMV) สามารถพบได้ในทุกภูมิภาค ซึ่งพบความชุกได้ตั้งแต่ 50-90% ความหลากหลายของ glycoprotein B (gB) ใช้ในการแยกลักษณะทางพันธุกรรมของ HCMV อย่างไรก็ตามข้อมูลลักษณะทางพันธุกรรม gB ของ HCMV ในประเทศไทยยังไม่ทราบแน่ชัดโดยเฉพาะอย่างยิ่งในเด็ก*

วัสดุและวิธีการ: *การศึกษาแบบตัดขวางมีวัตถุประสงค์เพื่อประเมินการติดเชื้อ HCMV ในเด็กกำพร้าที่ติดเชื้อ และไม่ได้ติดเชื้อเอชไอวีจำนวน 236 คน ในสถานรับเลี้ยงเด็กกำพร้านนทบุรี ประเทศไทย โดยนำปัสสาวะมาตรวจหาจีนจีบีของเชื้อ HCMV ใช้เทคนิค nested-PCR ใช้ restriction fragment length polymorphism (RFLP) และเทคนิคการหาลำดับเบสของดีเอ็นเอ เพื่อแยกลักษณะทางพันธุกรรม*

ผลการศึกษา: *61% (144/236) ของตัวอย่างตรวจพบเชื้อ HCMV โดยพบ 66.1% (37/56) ในเด็กติดเชื้อเอชไอวีและ 59.4% (107/180) ในเด็กที่ไม่ได้ติดเชื้อเอชไอวี การวิเคราะห์พหุตัวแปรพบว่าเด็กที่อาศัยอยู่ในห้องหนึ่งมีความเสี่ยงต่อการติดเชื้อ HCMV การวิเคราะห์พันธุกรรมพบว่าสายพันธุ์ที่พบมากที่สุดในเด็กเหล่านี้เป็น gB1; 85.4% (111/130) ตามด้วย gB3; 4.6% (6/130) gB2 และ gB4 เท่ากันที่ 2.3% (3/130) การติดเชื้อหลายสายพันธ์พบได้ 5.4% (7/130) ของตัวอย่าง*

สรุป: *การติดเชื้อ HCMV โดยเฉพาะสายพันธ์ gB1 พบได้สูงในเด็กกำพร้าไทยเหล่านี้โดยเฉพาะอย่างยิ่งในเด็กที่อาศัย อยู่ในห้องหนึ่งเพื่อป้องกันการแพร่กระจายของเชื้อ HCMV ในสถานรับเลี้ยงเด็กกำพร้า ควรจะมุ่งเน้นเรื่องพฤติกรรม ด้านสุขอนามัยของพี่เลี้ยงที่ดูแลเด็ก*