

Seroprevalence of cytomegalovirus infection in Thai adults detecting by ELISA

Parvapan Bhattarakosol*

Monkol Sithidajporn* Pattarasinee Bhattarakosol**

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- Objective** : *To study the seroprevalence of cytomegalovirus (CMV) infection in Thai adults*
- Design** : *Cross-sectional study*
- Setting** : *Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand*
- Subjects** : *575 sera of normal healthy Thai adults from blood donors (Thai Red-Cross Society), undergraduate students (Chulalongkorn University), and pregnant women (Antenatal care clinic, Chulalongkorn Hospital)*
- Methods** : *All sera were determined for the presence of anti-CMV IgG and IgM antibodies by enzyme linked immunosorbent assay (ELISA)*
- Results** : *The seroprevalence of CMV infection in Thai adults was 94.3% (542/575). The percentage of CMV seropositivity was 97 (294/303) in blood donors, 86 (148/172) in undergraduate students and 100 (100/100) in pregnant women. The results of CMV antibodies were significant difference between sex (p -value < 0.05), i.e., male 91.2% and female 96.8%. Moreover, our results indicated that CMV seronegative was found predominantly in male undergraduate students between ages 15 and 25 years.*

* Department of Microbiology, Faculty of Medicine, Chulalongkorn University

**Department of Mathematics, Faculty of Science, Chulalongkorn University

Conclusion : *The high prevalence of CMV infection in Thai adults indicated the possibility of having CMV infection early in lives. The infection might occur via congenital or perinatal or postnatal infection because the infection in female was higher than in male especially marriage women and pregnant women were 100%.*

Key words : *CMV infection, Thai adults.*

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Chulalongkorn University, Bangkok 10330, Thailand.

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- วัตถุประสงค์ : เพื่อศึกษาหาความชุกของการติดเชื้อไซโตเมกกาโลไวรัสในคนไทย
- รูปแบบการวิจัย : การศึกษาแบบ *cross-sectional*
- สถานที่ทำการศึกษา : ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร ฯ
- ประชากรที่ศึกษา : ตัวอย่างน้ำเหลืองจำนวน 575 ตัวอย่างเก็บจากคนไทยที่มีสุขภาพดี โดยเก็บจากผู้บริจาคโลหิต สภากาชาดไทย นิสิตจุฬาลงกรณ์มหาวิทยาลัย และ หญิงตั้งครรภ์จากคลินิกหญิงมีครรภ์ โรงพยาบาล จุฬาลงกรณ์
- วิธีการศึกษา-วัดผล : ทำการตรวจหาแอนติบอดีจำเพาะต่อเชื้อไซโตเมกกาโลไวรัสชนิด *IgG* และ *IgM* จากตัวอย่างน้ำเหลืองโดยวิธีอิมมูโนฟลูออเรสเซนซ์
- ผลการศึกษา : ความชุกของการติดเชื้อไซโตเมกกาโลไวรัสในคนไทยพบร้อยละ 94.3 (542/575) จำนวนผู้มีแอนติบอดีจำเพาะต่อไซโตเมกกาโลไวรัส คิดเป็นร้อยละ 97(294/303) ในผู้บริจาคโลหิต, ร้อยละ 86 (148/172) ในนิสิตจุฬาลงกรณ์มหาวิทยาลัย, และ ร้อยละ 100 (100/100) ในหญิงมีครรภ์ การติดเชื้อไซโตเมกกาโลไวรัสมีความแตกต่างกันในแต่ละเพศอย่างมีนัยสำคัญทางสถิติ ($p\text{-value} < 0.05$) คือ ในเพศชายพบร้อยละ 91.2 และเพศหญิงพบร้อยละ 96.8 นอกจากนี้ผลการศึกษายังชี้ให้เห็นว่า กลุ่มที่ยังคงปลอดการติดเชื้อส่วนมากพบในกลุ่มนิสิตมหาวิทยาลัยเพศชาย อายุระหว่าง 15 ถึง 25 ปี.

วิจารณ์และสรุปผล : คนไทยมีความชุกของการติดเชื้อไซโตเมกาโลไวรัสสูง ซึ่งมีความเป็นไปได้ว่าการติดเชื้อจะเกิดขึ้นในช่วงแรกของชีวิต โดยอาจเกิดขึ้นระหว่างมารดาตั้งครรภ์ หลังคลอดหรือระยะเลี้ยงดู เพราะการติดเชื้อในเพศหญิงพบสูงกว่าในเพศชาย โดยเฉพาะในผู้หญิงที่แต่งงานและตั้งครรภ์บ่อยละ 100

Cytomegalovirus (CMV) or human herpesvirus - 5 (HHV-5) is a large enveloped double - stranded DNA virus belonging to the family Herpesviridae and subfamily Gammavirinae. ⁽¹⁾ CMV can infect humans both vertically and horizontally. Sources of virus include oropharyngeal secretions, urine, cervical and vagina secretions, semen, breast milk, tears, feces, and blood. ⁽¹⁾ Spread within susceptible population or seronegative persons is enhanced by the prolonged secretion of infectious virus following primary infection. Moreover, intermittent shedding of CMV can be found in recurrent seropositive persons. ⁽²⁾ Mostly, CMV infection does not cause any serious symptoms to immunocompetent patients except in seronegative or immunocompromised hosts such as newborn infants, transplant patients, and immunodeficiency patients. ⁽¹⁻⁴⁾ At present, numbers of patients with HIV infection are increasing world wide, including Thailand. CMV recurrent infection or reactivation in those HIV patients can easily happen because of low or lost of immunity and the severity of infection might be manifested as CMV retinitis, colitis, encephalitis and other CMV related diseases. ⁽⁵⁻⁷⁾ Moreover, CMV can cause congenital

infection, perinatal and postnatal infection. The infected infants show clinical manifestations such as hepatomegalovirus, icterus, petechiae and involvement of central nervous system. Fetus infected *in utero* will suffer from microcephaly, cerebral calcifications, choreoretinitis, hepatospleenomegaly with jaundice, purpuric lesions, deafness and long bone structure changes. ⁽⁸⁻¹¹⁾ In this study, the prevalence of CMV infection in Thai adults was determined by detecting anti-CMV IgG and IgM by means of ELISA.

Materials and Methods

Serum specimens

A total of 575 sera were collected from healthy Thai adults. They were from 303 blood donors at the National Blood Centre, Thai Red Cross Society in May 1997 (Group 1), 72 Chulalongkorn University undergraduate students in May 1995 (Group 2) and 100 students in May, 1997 (Group 3), and 100 pregnant women who attended the antenatal care clinic of Chulalongkorn Hospital in May 1997 (Group 4). Their characteristics were clarified in Table 1. In group 1, 32 women had historical record of married status

Table 1. Characteristics of studied population.

Group	1*	2	3	4	Total
Male	184	24	52	0	260
Age (yrs)	16-51	20-23	17-25		16-51
Mean±SD	28.8±7.6	20.8±1.0	20.3±1.9		26.3±7.5
Female	119	48	48	100	315
Age (yrs)	17-56	17-25	18-25	15-40	15-56
Mean±SD	28.9±8.3	20.9±1.4	20.3±1.7	26.1±5.9	25.5±7.0
Total	303	72	100	100	575
Age (yrs)	16-56	17-25	17-25	15-40	15-56
Mean±SD	28.8±7.9	20.9±1.3	20.3±1.8	26.1±5.9	25.9±7.3

*The number represents group of studied population, i.e., blood donors; group 1, undergraduate students; group 2 (1995) and group 3 (1997), and pregnant women; group 4.

Detecting Anti-CMV IgG and IgM antibodies

CMV infection was determined by detecting CMV antibodies both IgG and IgM using Enzygnost Anti-CMV/IgG+IgM purchased from Behring, Germany. It is an enzyme immunoassay based on the indirect test principle. CMV antibodies (IgG+IgM) in the sample bind to the antigen-coated surface of the wells of the test plate. Then the anti-human IgG+IgM peroxidase conjugate binds to those immunocomplexes. After adding the substrate, an enzymatic reaction will result in colour production and sulphuric acid is added to terminate the reaction. The OD result is read at 450 nm within one hour. The cut-off value was calculated according to manufacturer's recommendation. The positive result means that person had CMV infection which probably be either recent infection or past infection.

Statistical analyses

The SPSS computer program was used throughout this study. Analysis of Variance (ANOVA) based on a cross-factor experimental design, randomized block design, and homogeneity of variance was done using the Least Square Difference (LSD) method.

Results

A total of 575 Thai adults with an age range between 15 to 56 years were tested for the presence of CMV infection by detecting anti-CMV (IgG+IgM) antibodies in sera using ELISA method. In this study, 3 different populations were done. Group 1 was the blood donors consisting of many occupations such as students, monks, military, business, and general work. This group represented general Thai adult

population. Group 2 and 3 were Chulalongkorn University undergraduate students collected in May 1995 and May 1997, respectively. Although they were from different years, these two groups could be analysed as single group because they were shown to be the same homogeneity of age (p -value > 0.05). Group 4 was pregnant women who attended antenatal care clinic at Chulalongkorn hospital. Among all groups, they were tested statistically significant different in age (p -value < 0.05). When all sera were analysed for the presence of anti-CMV IgG+IgM antibodies, 542 out of 575 (94.3%) were CMV antibody positive (Table 2). The percentage of CMV seropositive was 97 (294/303) in blood donors; Group 1, 88.9 (64/72) and 84 (84/100) in undergraduate students; Group 2 and 3, respectively and 100 (100/100) in pregnant women; Group 4 (Table 2). The CMV antibodies result does not depend on age (p -value > 0.05) but was found significant different between sex (male and female), i.e., male 91.2% vs female 96.8% (p -value < 0.05). The prevalence of CMV seropositivity in the undergraduate students group (86%; 148/172) was lower than the other 2 groups especially in male when it was compared to female of the same age (Group 2, 79.2% vs 93.8% and Group 3, 76.9% vs 91.7%, Table 2).

Discussion

CMV infection is known to spread worldwide. Transmission of CMV infection occurs via many different routes. Therefore, the prevalence of infection usually varies depending upon different groupings and differing socioeconomic status of the population. In most developed countries, infection increases steadily after infancy with a more rapid increase at school entry;

40-80% of children are infected before puberty and approximately 90-100% of population are infected during childhood.^(1,12) Up to 30,000-40,000 infants are born with this virus in the United States each year and only 10 per cent of infected infants show clinical manifestations.^(8,9) In this present study, the prevalence of CMV infection in Thai adults was subdivided into three different groups, i.e., blood donors, undergraduate students and pregnant women. The results showed that the percentage of CMV seropositivity was different among each group, i.e., 97 in the blood donors, 86 in the undergraduate students (combined Groups 2 and 3;

148/172) and 100 in the pregnant women (Table 2). The highest percentage was found among the pregnant women while the lowest was among the undergraduate students. Analysis of the data by distribution of sex and age range was conducted and indicated that infection rates in males were lower than among females (Group 1, 96.7% vs 97.5%; Groups 2+3, 77.6% vs 92.7%). Distribution of CMV infection between age ranges was the same when the 575 total samples were analysed (p-value > 0.05). According to our data, seronegative persons were found in 5.7% (33/575) of all studied Thai adults. Twenty seven out of 33 (82%)

Table 2. Seroprevalence of anti-CMV IgG+IgM among different groups of Thai adults comparing between age and sex.

Age range (yrs)	Group									
	Anti-CMV positive/negative cases (% positive)									
	1*		2		3		4		Total	
	M**	F	M	F	M	F	F	M	F	
15 - 25	78/2 (97.5)	50/1 (98)	19/5 (79.2)	45/3 (93.8)	40/12 (76.9)	44/4 (91.7)	50/0 (100)	137/19 (87.8)	189/8 (95.9)	
26 - 35	67/1 (98.5)	39/1 (97.5)	-	-	-	-	42/0 (100)	67/1 (98.8)	81/1	
36 - 45	27/2 (93.1)	23/1 (95.8)	-	-	-	-	8/0 (100)	27/2 (96.9)	31/1	
46 - 56	6/1 (85.7)	4/0 (100)	-	-	-	-	-	6/1 (100)	4/0	
Total	178/6 (96.7)	116/3 (97.5)	19/5 (79.2)	45/3 (93.8)	40/12 (76.9)	44/4 (91.7)	100/0 (100)	237/23 (91.2)	305/10 (96.8)	
Sum	294/9 (97.0)		64/8 (88.9)		84/16 (84)		100/0 (100)		542/33 (94.3)	

* The number represents group of studied population, i.e., blood donors; group 1, undergraduate students group 2 (1995) and group 3 (1997), and pregnant women; group 4.

**M; male and F; female

seronegative cases were in the age range of 15-25 years and 24 of those (88.9%) were undergraduate students (Table 2). This is probably because the undergraduate students, especially from Chulalongkorn University, were in the middle and upper economic class levels. The percentage of CMV seropositive which probably more accurately represent the Thai adult population would be the 97 % found in our blood donors because they were from many occupations and with a wide age range. CMV infection can also transmit via blood transfusion and only 1-5% of seronegative recipients exposed to seropositive blood can get infection.⁽¹⁾ The interesting data was the finding of 100 % prevalence of CMV infection in Thai pregnant women and in 32 female blood donors who were married. The vertical transmission of CMV infection occurred in about 40% of the studied pregnant women with primary infection, however, it also occurs in the presence of previous maternal immunity after recurrence of infection which transmission rates range from 0.2-2%.^(1,2,13) The CMV infection can be transmitted during their childhood from seropositive mothers via body secretions such as breast milk, tears, oropharyngeal secretion, etc.⁽¹⁾ According to the study of Tantivanich and Prasertsiriroje in 1981,⁽¹⁴⁾ reported a 30-35 % CMV seroprevalence rate in adults aged 18-50 years. It was quite low even though the studied population was the same group as in our work; that was blood donors from the Thai Red-Cross Society. In addition, our group also found a CMV seroprevalence rate of 71% in infants over 6 months old.⁽¹⁵⁾ Therefore, CMV infection in Thai children may occur very early in their lives. Unfortunately, our data could not evaluate the

transmission rate of CMV to fetuses and infants, however, the possibility of CMV infection might be very high because 100% of the studied pregnant women had CMV antibodies.

In conclusion, this study clearly showed that CMV seroprevalence in Thai adults changed during the past 16 years from 35% up to 94.3%. The seronegativity was found mostly in young adults (15-25 years) and predominantly in the males.

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