SEROPREVALENCE OF LATENT CYTOMEGALOVIRUS INFECTION AMONG ELDERLY THAIS

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Abstract. We determined the seroprevalence of latent cytomegalovirus (CMV) infection among young and elderly adults to test for a change in seroprevalence with increasing age. Thirty-two young and 32 elderly adults were tested for anti-CMV IgG, T-cell subgroup analysis, mental status and daily life activity. There was no significant difference in the seroprevalence of CMV infection between the two groups (59.4% *vs* 50.0%; p=0.616). The subgroup analysis of T-cells showed significantly lower percentages of CD3 (64.5±9.5% *vs* 69.0±4.5%; p=0.019) and CD8 (20.6±7.3% *vs* 28.3±6%; p<0.001), and a higher percentage of CD4 (42.2±7.5% *vs* 36.5±4.7%; p=0.001) cells in the elderly group. No differences in T-cell subgroups were observed by CMV serostatus subgroup among the young and elderly adult groups. Among the elderly there was a significantly lower mental status examination score among CMV positive subjects than CMV negative subjects (27.7 and 28.8 respectively, p=0.049), but no difference in the advanced daily life activity index between the two groups. These results suggest the prevelence of CMV infection in elderly Thais does not increase with age and is not associated with immune status; however, the presence of latent CMV infection in the elderly may be associated with a decline in mental status, but not the inability to carry out activities of daily living. Further studies with large number of patients are needed to explore these findings.

Keywords: cytomegalovirus, seroprevalence, T-cell, cytomegalovirus latent infection

INTRODUCTION

Cytomegalovirus (CMV), family Herpesviridae, is transmitted from person to person via saliva, urine, vaginal secretions, seminal fluid, breast milk and blood (Herndler-Brandstetter *et al*, 2006). CMV infection can cause severe congenital infections and illness among immunocompromised patients, such as those with AIDS or post-transplant patients, while in normal adults CMV infection is asymptomatic or causes mild mononucleosis like symptoms (Wreghitt *et al*, 2003). CMV is of interest because of its indirect effects (Freeman, 2009). It acts as an immune modulator causing inflammation and immunosuppression especially immunosenescence, which is a state of dysregulated immune function

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Table 1
Demographic and basic laboratory data of study subjects.

Variables	Young (*n*=32)	Elderly (*n*=32)	*p*-value
Mean age	28.7±4.7	68.3±7	<0.001
Male : Female	10 (31.3%):22 (68.7%)	14 (43.8%):18 (56.2%)	0.439
Occupation			
Agricultural worker	19 (59.4%)	1 (3.1%)	<0.001
Labor	0	2 (6.3%)	1
House wife	2 (6.2%)	1 (3.1%)	1
Factory worker	6 (18.8%)	17 (53.1%)	0.009
Officer	3 (9.4%)	0	1
Student	0	5 (15.6%)	1
Unemployed	2 (6.2%)	6 (18.8%)	0.257
Income per year (Baht/USD)			
No income	3 (9.4%)	1 (3.1%)	0.606
≤10,000/333	0	1 (3.1%)	0.317
10,000-100,000/333-3,333	5 (15.6%)	4 (12.5%)	0.719
≥100,000/3,333	24 (75.0%)	26 (81.3%)	0.762
Years of education	12.9±3.5	12.9±4.4	0.975
BMI (kg/m^2)	22.9±4.1	24.4±2.8	0.094
Hb (g/dl)	13.4±1.5	13.6±1.0	0.652
WBC (x1,000 l)	7.1±1.9	6.4±1.4	0.089
Lymphocyte count (%)	31.5±7.8	32.5±6.6	0.579
Absolute lymphocyte count (x1,000 l)	2,150.1±482.2	2,042±548.2	0.405
Platelet count (x1,000 l)	273.8±57.4	247.9±43.2	0.046
Albumin (g/dl)	4.6±0.3	4.4±0.3	0.002

that contributes to increased susceptibility to infection among the elderly (Pawelec and Larbi, 2008).

Immunosenescence is characterized by decreasing numbers of T-cell lymphocytes becoming dysfunctional by shifting from naïve T-cells to memory T-cells (Pawelec and Larbi, 2008). Several studies have found chronic antigen stimulation, especially with CMV latent infection, leads to dysfunction of T-cells (Herndler-Brandstetter *et al*, 2006; Vasto *et al*, 2007; Pawelec and Larbi, 2008). Unfortunately, there are limited studies of the prevalence of latent CMV infection and immunosenescence in Thailand and other developing countries. In this cross sectional study we aimed to assess the seroprevalence of latent CMV infection among healthy young and elderly adult Thais.

MATERIALS AND METHODS

Thirty-two adults aged 20-40 years and 32 adults aged ≥60 years were recruited for the study from the community in Bangkok, Thailand. Exclusion criteria for the study were a positive anti-HIV antibody test, pregnancy, the presence of concurrent infection (such as an upper respiratory infection or diarrhea) during study period, dependent people, and health care personnel/ ex- health care personnel, including day care workers for children or the elderly.

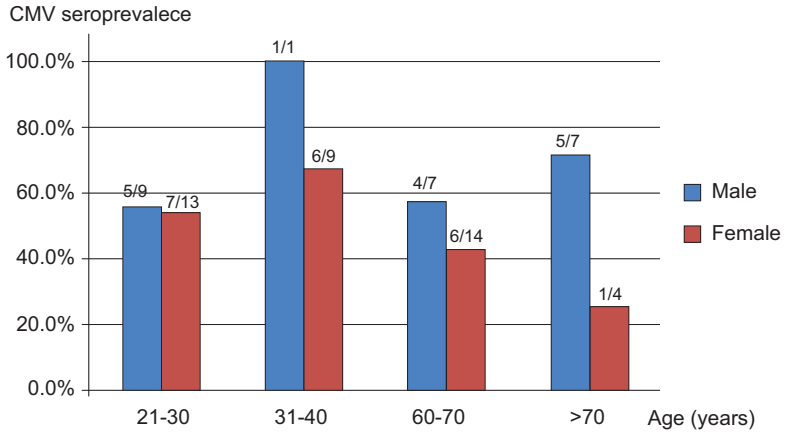
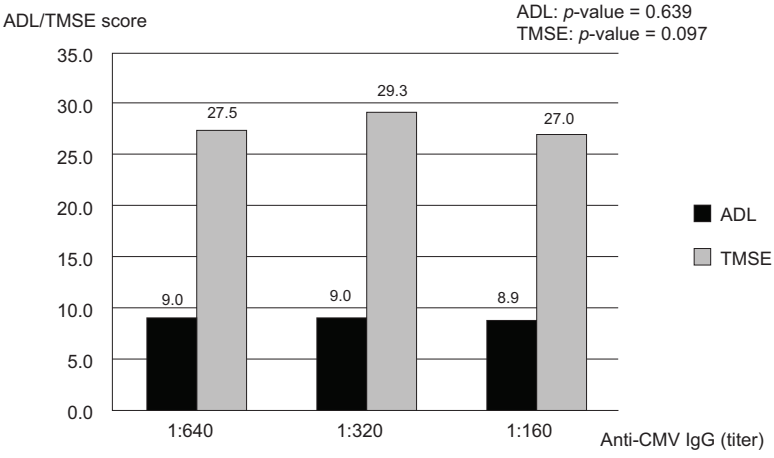


Fig 1–Seropvalence of latent CMV infection by age and sex.



ADL, advanced daily life activities (range 1-9)
TMSE, Thai mental status examination (range 1-30)

Fig 2–Anti-CMV IgG titer and ADL index/TMSE score.

Each participant gave written informed consent prior to participation. The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University.

Latent CMV infection was defined as a positive titer for anti CMV IgG detected by an in-house ELISA technique; the cut-off titer was set at ≥1:160. Laboratory tests obtained were: a complete blood count, the percentage of CD3 T-cells, CD4 T-helper cells, CD8 cytotoxic T-cells and a CD4/CD8 ratio. Elderly participants were tested for functional status with the Chula Advanced Daily Life Activity (ADL) Index (Jitapunkul *et al*, 1994) and cognitive function was assessed with a Thai mental status examination (TMSE) (Train the brain forum committee, 1993).

A chi-square test was used to compare the seroprevalence of latent CMV infection between groups and a paired-sample *t*-test was used for continuous variables. A statistical difference was considered as a p-value ≤ 0.05.

RESULTS

The demographic and basic laboratory data are shown in Table 1. The average platelet count and albumin levels were significantly lower in the elderly group (p=0.046 and p=0.002, respectively). The other demographic and laboratory data were not significantly different from each other. The seroprevalence of CMV was 59.4% in the young adult group and 50% in the elderly group (p=0.616). The seroprevalences by age and sex are shown in Fig 1. The associations between sex, age and CMV serostatus on nutritional status and blood parameters are shown in Table 2.

Table 2
Effects of sex, age and CMV serostatus on nutritional status, blood parameters and functional status.

Variables	Sex		*p*-value	Age		*p*-value	CMV		*p*-value
	Female	Male		Young adult	Elderly		Positive	Negative	
BMI	23.4±3.8	24.1±3.3	0.473	22.9±4.1	24.4±2.8	0.094	23.9±3.9	23.4±3.3	0.584
Hb (g/dl)	12.9±0.8	14.5±1.3	<0.001**	13.4±1.5	13.6±1.0	0.652	13.5±1.2	13.5±1.3	0.921
Total lymphocyte count (x1,000 l)	2,098.7±450.8	2,091.6±618.1	0.958	2,150.1±482.2	2,042.0±548.2	0.405	2,092.3±518.0	2,100.5±520.5	0.95
CD3 %	67.5±7.0	65.5±8.9	0.334	69.0±4.5	64.5±9.5	0.019	66.3±7.9	67.4±7.7	0.575
CD4 %	39.7±6.9	38.9±6.9	0.655	36.5±4.8	42.2±7.5	0.001**	38.9±6.5	40.0±7.4	0.512
CD8 %	24.7±7.3	24.0±8.5	0.721	28.3±6.0	20.6±7.3	<0.001**	24.8±8.8	23.9±6.2	0.632
CD4/CD8 ratio	1.8±0.7	2.0±1.2	0.451	1.4±0.4	2.3±1.0	<0.001**	1.8±0.9	1.9±0.9	0.906
Albumin level	4.5±0.3	4.6±0.3	0.149	4.6±0.3	4.4±0.3	0.002**	4.5±0.3	4.6±0.3	0.271

Values expressed as mean ± SD. ** Indicates statistically significant ($p<0.01$).

There were no significant differences in lab values between sexes except females had a lower average hemoglobin level than males (12.91±0.79 and 14.45±1.23 g/dl, respectively ($p<0.001$). Significantly lower percentages of CD3 and CD8 and a significantly higher percentage of CD4 cells were seen in the elderly group. The mean CD4/CD8 ratios were 2.3 and 1.4 in the elderly and young adult groups, respectively ($p<0.001$). There were no significant differences in body mass index (BMI) by age group but the mean albumin level was lower in the elderly group than the young adult group (4.4±0.25 and 4.63±0.031 g/dl, respectively) ($p=0.002$). There were no significant differences in BMI, albumin, T-cell lymphocyte counts or ADL index results by CMV serostatus. The TMSE was statistically lower in the CMV positive group than the CMV negative group (27.7 and 28.8, respectively) ($p=0.049$) (Table 3).

Evaluating risk factors for CMV infection revealed no differences in history of blood transfusion, intravenous drug use (IVDU), obtaining a tattoo or sexual activity (number of sexual partners) (data not shown) by CMV serostatus.

The anti-CMV IgG titer was not significantly related to the ADL index or TMSE score (Fig 2).

DISCUSSION

CMV infection is common worldwide and affects 60-100% of the adult population (Herndler-Brandstetter *et al*, 2006), depending on the region, with a reported increase in prevalence with age (Looney *et al*, 1999; Wikby *et al*, 2001). The seroprevalences of CMV in New York, US (Looney *et al*, 1999) among healthy elderly (aged ≥ 60 years) and other adults (aged < 60 years) were 81% and 42%, respec-

Table 3
Laboratory factors associated with CMV status and age group.

Variables	Young (*n*=32)		*p*-value	Elderly (*n*=32)		*p*-value
	CMV + (*n*=19)	CMV - (*n*=13)		CMV + (*n*=16)	CMV- (*n*=16)	
BMI (kg/m^2)	23.6±4.6	21.9±3.2	0.243	24.2±2.8	24.6±2.9	0.679
Hb (g/dl)	13.5±1.4	13.2±1.6	0.593	13.5±0.9	13.7±1.1	0.597
WBC (x1,000 l)	7.2±2.3	7.0±1.2	0.749	6.2±1.3	6.5±1.6	0.490
%L	30.4±7.6	33.0±8.0	0.373	34.6±6.9	30.4±5.8	0.072
Total lymphocyte (x1,000 l)	2,083.6±502.8	2,247.3±452.0	0.354	2,102.7±552.0	1,981.2±555.4	0.539
Platelets (x1,000 l)	280.2±64.0	264.4±47.1	0.453	242.3±47.9	253.5±38.7	0.473
CD3 %	68.6±5.1	69.6±3.5	0.549	63.4±9.7	65.5±9.6	0.544
CD4 %	36.4±4.9	36.8±4.7	0.819	41.8±7.0	42.6±8.3	0.766
CD8 %	28.5±7.2	27.9±4	0.803	20.5±8.7	20.6±5.8	0.962
CD4/CD8 ratio	1.4±0.5	1.4±0.3	0.888	2.4±1.1	2.3±0.9	0.774
Albumin (g/dl)	4.6±0.3	4.7±0.3	0.469	4.3±0.3	4.5±0.2	0.143
ADL (max =9)	-	-	-	8.9±0.3	9.0±0.0	0.333
TMSE (max=30)	-	-	-	27.7±1.7	28.8±1.1	0.049*

Values expressed as mean ± SD. *Indicates statistically significant $p<0.05$. ADL, advanced daily life activities with the Chula ADL Index; TMSE, Thai mental status examination

tively. In Swedish individuals aged 86-92 years (Olsson *et al*, 2000), the seroprevalence was 90%, compared to 67% among adults aged 40-60 years.

A previous study of latent CMV infection among healthy Thais revealed a prevalence of 52.38% among 18-55 year old blood donors in 1998 (Amarapal *et al*, 2001), 71.8% among various age groups attending 4 Thai hospitals in 1997 (Tantivanich *et al*, 1999) and 94.3% among blood donors, students and pregnant women in 1995-1997 (Bhattarakosol *et al*, 1998).

In our study, the prevalences of CMV infection were 59.4% among healthy young adults (aged 20-40 years) and 50% among healthy elderly adults (≥60 years). This difference was not statistically significant, p=0.616. These results are similar to those reported by Bhattarakosol *et al* (1998), whose study failed to demonstrate an increase in the seroprevalence of CMV with increasing age. These results might be explained by the fact that Thai children have already contracted CMV prior to adulthood. In a study from Thailand and by Tantivanich *et al* (1999) the highest seropositive rate was among subjects aged 3-6 years. In another study from Thailand by Likitnukul *et al* (2003) the reported CMV prevalence was 75% among children aged 37-79 months.

In studies from other countries, Bate *et al* (2010) found no significant increase in CMV seroprevalence with age and Lübeck *et al* (2010) found biphasic peaks (childhood and young adulthood) in CMV seroprevalence. Many factors that influence CMV infection, such as breast feeding, sexual behavior and crowding, change with time. Therefore, cross sectional studies such as those by Bate *et al*

(2010), Lübeck *et al* (2010) and our study cannot provide explanations for these findings.

Total lymphocyte counts, T-cell subsets (CD4, CD8) and CD4/CD8 ratios are influenced by ethnicity, sex, age, physical stress and a variety of infectious agents, such as HIV infection (Uppal *et al*, 2003). A previous study among healthy 20-49 year old Thais showed lower CD3 and CD4 levels and CD4/CD8 ratios than those among caucasians and no difference between age groups (Vithayasai *et al*, 1997). In our study, a statistically significant increase in CD4 counts and decrease in CD8 counts resulted in an increase in CD4/CD8 ratios with increasing age without clinical significance. To explain these findings further studies are needed. There were no significant differences in CMV serostatus by sex. Our results are different from those of Looney *et al* (1999) who found latent CMV infection which was independent of age but had a significant effect on T-cell subsets.

Previous studies have found factors related to higher mortality among the elderly called "the immune risk phenotype" (IRP): a CD4:CD8 ratio <1, poor T-cell proliferation due to mitogens, increased CD8+CD28-CD57+ cells, low numbers of B cells, CMV seropositivity, clonal expansions of CD8 cells carrying receptors for CMV or Epstein-Barr virus (EBV) antigens and a high proportion of dysfunctional cells among CMV-specific CD8 cells (Birgit *et al*, 2007; Vasto *et al*, 2007). Latent CMV infections take part in immunosenescence by decreasing numbers of naïve T-cells while simultaneously accumulating greater numbers of effector T-cells (CD8+CD28-) (Birgit *et al*, 2007). Our study did not differentiate T-cell subjects; therefore, we cannot explain the aging effect on T-cells.

A prospective cohort study by Aiello *et al* (2006) demonstrated an increase in CMV IgG antibody levels is associated with a more rapid cognitive decline assessed by mental status examination. An association between latent CMV infection and the prevalence of frailty was described by Schmaltz *et al* (2005). In their study, there were significantly lower mental status examination scores among CMV positive elderly, but no association with anti-CMV IgG titer. The lower TMSE scores in this study may be confounded by lower education levels. CMV positive elderly subjects had fewer years of education than CMV negative persons (11±5 and 15±4 years, respectively) (p=0.021).

There were severed limitations in this study. Our sample size was small making it difficult to evaluate some variables. We evaluated T-cell subsets by flow cytometric analysis; specific types of T-cells were not analyzed. More sensitive tests to evaluate cognitive decline and frailty were not conducted. Further studies regading CMV are needed to explore transmission, prevention, immunosenescence and immunomodution in Thailand.

In conclusion, latent CMV infection did not increase with age among elderly Thais.

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