

A Study of Cytomegalovirus Serology among HIV-Infected Patients in the Highly Active Antiretroviral Therapy Era

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Abstract

Cytomegalovirus (CMV) is one of the most common opportunistic viruses in human immunodeficiency virus (HIV)-infected patients. The aim of this study was to determine the CMV seroprevalence among HIV-infected patients and investigate the correlation between the CMV immunoglobulin G (IgG) antibody titers and cluster of differentiation 4 (CD4) T-cell counts, as well as highly active antiretroviral therapy (HAART) use. Serum samples from 69 HIV-infected patients and 65 HIV-seronegative persons attending Dr. Hasan Sadikin General Hospital Bandung in March–June 2012 were examined to detect CMV IgG antibody using electrochemiluminescence immunoassay (ECLIA). Data were analyzed using chi-square test, t-tests and analysis of variance (ANOVA). The results show that there were no statistically significant differences in the seroprevalence of CMV between HIV-infected (97%) and HIV-seronegative persons (94%). The mean of CMV IgG antibodies titers in HIV-infected patients (335.39+174.87 U/mL) were significantly higher than that of HIV-seronegative persons (240.59+192.76 U/mL). There was no significant correlation between CMV IgG antibody titers and CD4 T-cell counts (the mean was 393.58+209.22 cells/mm³). The titers of CMV IgG antibodies were significantly inversely associated with HAART use. The mean of CMV IgG antibody titers in HIV-infected patients on HAART (335.41+172.98 U/mL) were significantly higher than patients without HAART (204.8+213.91 U/mL). In conclusions, this study confirms a high seroprevalence of CMV among HIV-infected patients. High titers of CMV are inversely associated with HAART use while no correlation with CD4 T-cell counts was found. [MKB. 2013;45(2):112–7]

Key words: CD4, *Cytomegalovirus* (CMV), HAART, HIV, IgG

Studi Serologi *Cytomegalovirus* pada Pasien yang Terinfeksi HIV di Era *Highly Active Antiretroviral Therapy*

Abstrak

Cytomegalovirus (CMV) adalah salah satu virus oportunistik yang paling umum pada pasien yang terinfeksi *human immunodeficiency virus* (HIV). Tujuan penelitian ini untuk mengetahui seroprevalensi CMV pada pasien HIV dan meneliti korelasi antara titer antibodi imunoglobulin G (IgG) CMV dan jumlah sel-T *cluster* diferensiasi 4 (CD4) serta penggunaan *highly active antiretroviral therapy* (HAART). Sampel serum dari 69 pasien HIV dan 65 HIV-seronegatif yang berkunjung ke Rumah Sakit Dr. Hasan Sadikin Bandung pada bulan Maret–Juni 2012 diperiksa untuk mendeteksi antibodi IgG CMV dengan *immunoassay electro chemiluminescence* (ECLIA). Data dianalisis dengan menggunakan uji chi-kuadrat, t, dan *analysis of variance* (ANOVA). Hasil penelitian menunjukkan tidak ada perbedaan yang signifikan antara seroprevalensi CMV pada pasien HIV (97%) dan HIV-seronegatif (94%). Titer antibodi rata-rata IgG CMV pasien HIV (335,39+174,87 U/mL) signifikan lebih tinggi daripada HIV-seronegatif (240,59+192,76 U/mL). Tidak ada hubungan yang signifikan antara titer antibodi IgG CMV dan jumlah sel-T CD4 (rata-rata 393,58+209,22 sel/mm³). Titer antibodi IgG CMV secara signifikan berhubungan terbalik dengan penggunaan HAART. Titer antibodi IgG CMV rata-rata pasien HIV dengan HAART (335,41+172,98 U/mL) signifikan lebih tinggi dibandingkan dengan pasien tanpa HAART (204,8+213,91 U/mL). Simpulan, penelitian ini menegaskan seroprevalensi CMV pasien HIV dan titer antibodi IgG CMV yang tinggi berhubungan terbalik dengan penggunaan HAART tetapi tidak berkorelasi dengan sel-T CD4. [MKB. 2013;45(2):112–7]

Kata kunci: CD4, *Cytomegalovirus* (CMV), HAART, HIV, IgG

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Introduction

The human cytomegalovirus (CMV) which is a beta-herpes virus is considered the most important viral opportunistic pathogen in patients with acquired immune deficiency syndrome (AIDS). Epidemiological studies showed that infections caused by CMV occur frequently across countries in the world and CMV seroprevalence in general population is estimated to range between 60% and 90% in developed countries, even higher rates (>90%) in developing countries.¹ Seroprevalence of CMV infection in human immunodeficiency virus (HIV)-infected patients is also high, ranging from 50% to above 90%.¹⁻³ Detectable CMV viral loads are associated with increased mortality, even in the era of highly active antiretroviral therapy (HAART).⁴

Although primary infection with CMV does not generally produce symptoms in healthy individuals, the infection may cause more serious and potentially life-threatening illnesses among HIV-infected patients. Cytomegalovirus retinitis is the most common serious ocular complication in HIV-infected patients, which occurs in 40% of AIDS patients.^{5,6} In addition, CMV infection causes diseases in several organ systems such as the central nervous system (CNS), gastrointestinal (GI) tract and pneumonitis.⁶⁻⁹

The majority of diseases relate to reactivation of latent infection. Most cases of CMV disease occur in HIV-infected patients with advanced immunosuppression. The HIV-infected patients with cluster of differentiation 4 (CD4) T-cell counts <100 cells/mm³ are at significant risk for CMV reactivation leading to invasive disease.¹⁰ Prior to the development of HAART, CMV disease occurred in 10.9% of patients with AIDS.⁶ The introduction of HAART has led to changes in the incidence of CMV diseases.⁵ It has been reported that CMV disease may occur during immune recovery in the HAART era.^{9,11,12}

Considering the above facts, this study was undertaken to assess the seroprevalence of CMV and to measure the titers of CMV IgG antibody against CMV and its association with CD4 and the use of HAART among HIV/AIDS patients. These data may have important implications with regard to the transmission of CMV.

Methods

The present study was carried out among 69 HIV-infected patients and 65 HIV-seronegative persons who visit a major referral hospital, Dr. Hasan Sadikin Hospital, Bandung, Indonesia. The HIV-seronegative persons were matched

to HIV-infected patients based on age and sex as a control group. The use of highly active antiretroviral therapy was recorded from medical records.

Blood samples were collected from all 134 study participants and sera were separated and stored at -20 °C until testing. The HIV status was confirmed by enzyme-linked immunosorbent assay (ELISA). The CD4 cell counts were measured by the Becton Dickinson fluorescence-activated cell sorter (BD FACSCount™) cytometer according to the manufacturer's instructions. Serum anti-CMV was determined by a commercially available test kit, the Elecsys CMV immunoglobulin G (IgG) assay (Roche Diagnostics) in accordance with the manufacturer's instructions. Positive and negative standard sera, accompanying the kit were included in each assay.

The results were then analyzed statistically using the statistical package for social sciences (SPSS) version 11.0 for Windows. The mean ± standard deviation (SD), median and ranges were calculated and proportion, as well as frequency tables were used to summarize the categorical variables. The student t-test was used to compare age distribution for both groups. Differences in CMV seropositivity rates among different groups were evaluated using the chi-square test. Mean titer levels were compared between HIV-infected and HIV-seronegative controls using two sample t-tests. The statistical significance of CMV IgG antibody titers with other associated factors (CD4 and HAART use) was obtained using univariate analysis of variance (ANOVA). The p value <0.05 was taken as the level of significance.

All participants gave written informed consent for participation. Ethical clearance was obtained from the Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital/Faculty of Medicine of Universitas Padjadjaran, Bandung, Indonesia.

Results

Overall, a total of 69 patients with HIV infection (34 males and 35 females) took part in the study. The age ranged from 22–55 years, with the mean age 32±5 years, median 32 years. The control group consisted of 34 HIV-seronegative males and 31 HIV-seronegative females with the mean age of 29.1±12.1 years (median 28 years, range 1–56 years). There was no significant statistical difference between HIV-infected patients and the control group in age ($p>0.05$) and gender distributions ($p>0.05$) (Table 1).

Out of 69 of the HIV-infected patients, 97% were seropositive for CMV IgG antibodies,

Table 1 Age and Gender of Study Participants

Characteristics	HIV-seronegative (n=65)	HIV-infected (n=69)	p
Age			
Mean+SD (range)	+5 (22–55)	31+10 (17–58)	>0.05
Median	32	29	
Gender			
Female	31	35	>0.05
Male	34	34	

Note: n=number of subjects; SD=standard deviation; *p<0.05, statistically significant

Table 2 The Titers of Cytomegalovirus IgG Antibody

Characteristics	HIV-seronegative (n=65)	HIV-infected (n=69)	p
Mean	240.59	335.39	>0.05
SD	192.76	174.87	
Median	170.7	403	

Note: n=number of subjects; SD=standard deviation; *p<0.05, statistically significant

slightly higher than the 94% in HIV-seronegative persons. The difference was not statistically significant (p>0.05). We found only 6% of HIV-seronegative persons and 3% of HIV-positive persons with a negative test result for CMV IgG antibody (Figure 1).

Immunoglobulin G antibodies against CMV are more frequently found in HIV-infected patients with a CD4 cell count of more than 500

cells/ mm³ (Figure 2). No significant correlation was observed between the CMV IgG antibody titers and CD4 counts (Table 3). However, the titers of CMV IgG antibodies were inversely associated with HAART use. It appears that the CMV IgG antibody titers in HIV-infected patients on HAART are higher than those of HIV-infected without HAART.

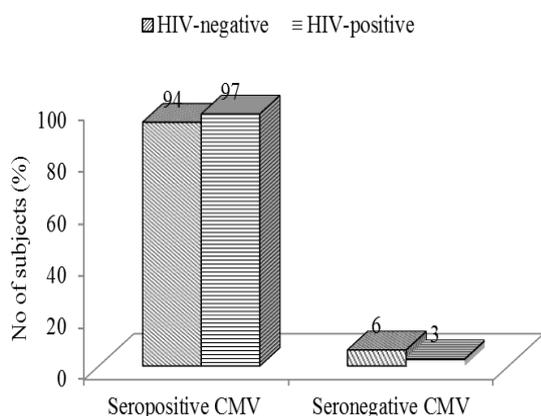


Figure 1 Seroprevalence of CMV among HIV-Infected Patients and HIV Seronegative Persons

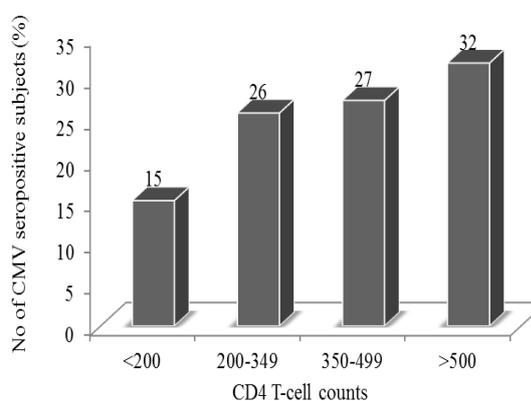


Figure 2 Distribution of CMV Seropositive by the Rate of CD4 T-cell Counts among HIV-Infected Patient

Tabel 3 The Titers of Cytomegalovirus IgG Antibody and CD4 T-cell Counts among HIV-Infected Patients

	The Titers of CMV IgG Antibody		p
	Mean (U/mL)	SD	
CD4 T-cell counts			
<200	264.15	184.87	>0.05
200–349	344.90	184.97	
350–499	332.21	161.90	
>500	364.77	176.07	
HAART use			
on HAART	335.41	172.98	<0.05
without HAART	197.87	188.14	

Note: SD=standard deviation; *p<0.05, statistically significant

Discussion

Our study demonstrates that IgG antibodies against CMV were high in both HIV-infected and HIV-seronegative persons and the difference between the two groups was not statistically significant. Our findings are consistent with earlier studies which found that there was no statistically significant difference between CMV seroprevalence in HIV-infected patients and HIV-seronegative persons.¹³ Cytomegalovirus is well known as an extremely common virus worldwide. The seroprevalence of CMV varies worldwide, and is related to geographic, ethnic and social factors.¹⁴ The reason for the high seroprevalence of CMV in general population is that CMV primarily spread through close interpersonal contact with infected blood and body fluids including saliva, urine, breast milk, or genital secretions. Cytomegalovirus is also able to cross the placental barrier. As a result, CMV can be transmitted in any way that involves passing secretions. This includes sexual contact, blood transfusion, organ transplant and breastfeeding. We found almost all HIV-infected patients (97%) had IgG antibodies to CMV. This study also confirms previous results showing the high seroprevalence of CMV IgG antibodies in HIV-infected patients. Interestingly, when we did an analysis to compare IgG antibody titers, the results showed significantly higher IgG antibody titers against CMV in HIV-infected patients compared to HIV-seronegative persons. The high seroprevalence of CMV in HIV-infected patients is related to sexual activity. A previous study has identified sexual activity and behaviors as important risk factors for acquiring CMV among HIV-infected patients. Another study also found that significant risk factor for CMV infections was

homosexual or bisexual orientation.¹⁵ Another study reported that the maximum prevalence of CMV antibody was seen in HIV-infected patients with unsafe sex and intravenous drug users (IDUs).³

It has been stated that CMV diseases are associated with a compromised immune system in HIV-infected patients. The disease typically occurs in HIV-infected patients with a CD4 count of <50 to 100 cells/mm³.¹⁶ A study indicated that insufficient help of CD4+ T cells in HIV-1–Infected patients may cause loss of control of CMV dissemination.¹⁰ However, it has also been reported that CMV disease occurred in patients with a CD4 count of >100 cells/mm³.¹⁷ The presence of CMV antibody does not appear to be associated with the clinical stage of HIV-1 infection, suggesting that a higher CD4 T-cell count does play a role in immunity against CMV infection. Our findings showed no significant correlation between the titer of all three viruses and CD4 cell counts. However, we can see that most HIV-infected patients who were CMV seropositive had a CD4 count of >500 cells/mm³.

Analysis of the data indicated a statistically significant inverse association between the titer of CMV IgG antibody and treatment of HIV-infected patients on HAART. We observed higher CMV IgG antibody titers in patients on HAART compared to those in patients without HAART. This higher cytomegaloviremia in HIV patients was associated with higher mortality during the pre-HAART era. However, in the HAART era, it has been reported that CMV disease may occur during immune recovery. Our data show that CMV IgG antibodies in HIV patients on HAART were detected more often in patients with a mean CD4 cell count of >500 cells/mm³. These findings suggest that higher titer of

cytomegalovirus IgG antibody after antiretroviral therapy may also be associated with immune restoration inflammatory syndrome (IRIS). It has been suggested that IRIS through HAART may be a key component of CMV infection. Immune restoration inflammatory syndrome may occur in HIV-infected persons, ranging from less than 10% to more than 50%, after starting HAART when there is a surge in reconstitution of effector and regulatory T cells.^{18,19} A prior study demonstrated that CMV retinitis after HAART is associated with increased plasma levels of IgG anti-CMV antibody.¹¹ The immunopathological process underlying IRIS is not fully understood yet. Therefore, it is suggested that the syndrome is the result of exaggerated and dysregulated cellular immune responses that depend on the associated pathogen.^{19,20} In viral infections such as infections caused by cytomegalovirus, CD8+ T cells in particular are involved in protection and immunopathology.^{11,20}

In conclusion, this study has identified that infection with CMV is very common both in general population and in HIV-infected patients. High titers of CMV among HIV-infected patients are inversely associated with HAART use but no correlation with the CD4 T-cell count is found. It is difficult to identify an acute CMV infection because the disease is almost always asymptomatic and usually presents nonspecific clinical signs. Nevertheless, the CMV has been known as poses major health problems as it may cause serious morbidity and mortality in HIV-infected patients. Hence, these findings underline the importance of prevention strategies to reduce CMV disease and CMV-associated immune recovery syndrome.

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