

The Prevalence of Cytomegalovirus Infection in Human Immunodeficiency-Virus-Infected Infants with Pneumonia

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Abstract

From September 1995 to January 1996, 15 HIV-infected infants with pneumonia and 15 non-HIV-infected controls were studied for the prevalence of cytomegalovirus infection at Chiang Mai University Hospital. The prevalence of CMV infection in HIV-infected infants who had pneumonia was higher than in non-HIV-infected infants as shown by CMV-PCR, CMV-IgM and CMV-IgG (73% vs 0%, 33% vs 7% and 80% vs 47% respectively). HIV-infected infants had active CMV infection more often than non-HIV-infected infants. PCR CMV is more sensitive than CMV-IgM in detecting active CMV disease, as shown in two cases who had histopathological evidence of CMV pneumonitis and a positive PCR CMV test but a negative CMV-IgM test. (*J Infect Dis Antimicrob Agents* 1997;14:93-6.)

INTRODUCTION

Cytomegalovirus (CMV) infection is common among human population throughout the world. Study on the seroprevalence of CMV infection have shown that 84 percent of the normal adult population in Thailand are antibody-positive for the virus (1). CMV is well known for its impact in causing disease in human-immunodeficiency-virus (HIV)-infected population. Study performed in HIV-infected children in New York and New Jersey revealed a CMV prevalence of 31-46 percent respectively (2,3). In Thailand the prevalence of CMV infection in HIV-infected children is not known.

Although most CMV infections in healthy individuals are subclinical, the virus can cause many forms of symptomatic illness, particularly in HIV-infected population. These manifestations include pneumonia, retinitis, colitis, hepatitis, neutropenia, thrombocytopenia, and, less

commonly, involvement of the CNS and adrenal glands. In this study we reported the prevalence of CMV infection in HIV-infected infants with pneumonia.

METHODS

Subjects

From September 1995 to January 1996, all HIV-infected infants whose ages were less than 1 year and who were admitted to the pediatric ward at Chiang Mai University Hospital because of severe pneumonia were included in the study. Non-HIV-infected control group of infants were enrolled simultaneously with the HIV-infected infants. Infants in the control group were selected to match those in the study group in term of age and time of admission. Infants in the control group with clinical manifestations of congenital or acquired CMV infection were excluded.

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Diagnosis of HIV infection

The diagnosis of HIV infection was made when the serum was repeatedly reactive by both ELISA (Enzymun-Test® Anti-HIV 1+2, Boehringer Mannheim GmbH Diagnostica) and particle-agglutination test (Serodia®-HIV, Fujirebio Inc., Tokyo, Japan) and symptoms and signs of HIV infection or HIV-related conditions were present, such as generalized lymphadenopathy, hepatomegaly, splenomegaly, and persistent oral candidiasis (4).

Diagnosis of CMV infection

Serum CMV-Ig M and CMV-IgG: Serum CMV-Ig M and CMV-IgG were performed according to the direction of manufacturer (Vironostika® anti-CMV-IgM, Vironostika® anti-CMV-IgG, Netherlands).

CMV DNA amplification by nested PCR method (CMV PCR): Plasma samples were collected from the EDTA blood. Then 100 µl of plasma was added to 100 µl of the lysing buffer (containing 100 mM KCL, 20 mM Tris-HCl pH 8.3, 5 mM MgCl₂, 0.2 mg/mL gelatin and 0.9 percent tween at the final concentration). Proteinase K was added to a final concentration of 60 µg/mL and the mixture was incubated for 60 minutes at 56°C and boiled for 10 minutes. The clear treated plasma was then analyzed by nested PCR method. PCR amplification was performed within the conserved region of the immediate-early gene. The outer primer set consisted of MG1, position 2333 to 2352 (5'-AGAGTCTGCTCTCCTAGTGT-3') and MG2, positions

2602 to 2621 (5'-CTATCTCAGACACTGGCTCA-3'). The inner primer set consisted of IE1, upstream primer (5'-CCACCCGTGGTGCCAGCTCC-3') and IE2, downstream primer (5'-CCCGCTCCTCCTGAGCACCC-3')(5). The treated plasma was amplified in a reaction mixture and then the reaction mixture was placed in the Thermal cycler, 40 cycles of 1 min at 94°C, 1 min at 55°C and 2 min at 72°C, using the outer and inner primers. The amplified PCR product is analyzed by electrophoresis in 2 percent agarose containing etidium bromide. The amplified positive sample is shown as DNA bands with 162 bp in length under UV illumination (6).

Statistical analysis

T-test and Chi-square analysis were used to compare the age and markers of CMV infection in the HIV infected infants and the non-HIV-infected infants.

RESULTS

A total of 15 HIV-infected infants with pneumonia and 15 non-HIV-infected controls were enrolled in the study. The characteristics and results of CMV-PCR, CMV-IgM, and CMV-IgG were demonstrated in Table 1. The median ages of 15 HIV-infected infants and 15 non-HIV-infected controls was 4 and 5 months respectively. Both groups had the same male to female ratio, that was 9:6. The age and sex of both group did not differ statistically (T-test for age and Chi-square test for sex).

Table 1. Characteristics and CMV markers of HIV-infected children and non-HIV-infected children.

HIV-infected children						non-HIV-infected children					
No.	Age (mo.)	Sex	PCR CMV	CMV IgM	CMV IgG	No.	Age (mo.)	Sex	PCR CMV	CMV IgM	CMV IgG
1	8	m	pos	neg	400	1	7	m	neg	neg	neg
2	2	f	pos	neg	200	2	2	m	neg	neg	neg
3	3	m	pos	100	200	3	4	m	neg	100	400
4	3	m	pos	>320	200	4	2	m	neg	neg	100
5	9	f	pos	1,600	800	5	8	f	neg	neg	400
6	3	m	pos	neg	100	6	2	f	neg	neg	200
7	4	m	pos	neg	100	7	5	m	neg	neg	neg
8	2	f	pos	100	200	8	2	m	neg	neg	neg
9	2	f	pos	neg	200	9	2	f	neg	neg	neg
10	7	m	pos	neg	100	10	7	m	neg	neg	800
11	4	m	pos	200	neg	11	5	f	neg	neg	neg
12	3	f	neg	neg	neg	12	4	f	neg	neg	neg
13	9	m	neg	neg	neg	13	10	m	neg	neg	1,600
14	11	m	neg	neg	1,600	14	10	f	neg	neg	200
15	4	f	neg	neg	100	15	6	m	neg	neg	neg
% positive			73	33	80	% positive			0	7	47

CMV-PCR was detected in 11 of 15 (73%) HIV-infected infants, 0 of 15 (0%) non-HIV-infected infants. CMV-IgM was detected in 5 of 15 (33%) HIV-infected infants, one of 15 (7%) non-HIV-infected infants and CMV-IgG was detected in 12 of 15 (80%) HIV-infected infants, 7 of 15 (47%) non-HIV-infected infants. All the CMV infection markers were found more often in HIV-infected infants than in non-HIV-infected infants (Chi-square test, $P < 0.05$). All 5 but one cases with positive CMV-IgM were also PCR CMV positive.

Lung histopathologic examinations of 6 HIV-infected infants who died were demonstrated in Table 2. Two of the four infants who had positive PCR CMV showed evidence of active CMV pneumonitis, one as the sole pathogen, the other as a co-infection with *Pneumocystis carinii*. The other two infants with positive PCR CMV and CMV-IgM were found to have histopathologic evidence of *Pneumocystis carinii* pneumonia. In two infants who were negative for both PCR CMV and CMV-IgM, there was no pathology suggestive of CMV infection in the lung.

DISCUSSION

This study showed that the prevalence of CMV infection in HIV-infected infants who had pneumonia was higher than in non-HIV-infected infants as shown by all these markers. By PCR CMV, it was also demonstrated that HIV-infected infants had active CMV infection more often than non-HIV-infected infants (73 % vs 0 %). The incidence of active CMV infection in infants in this study was higher (73%) than in the study of Frenkel LAD et al (9/22, 41%)(3) and in the study of Chandwani S et al (12/44, 27%) (2). However, in our study, only HIV-infected patient with pneumonia were included, whereas in the other two reports, all HIV-infected children were studied.

CMV infection can be recognized by many diagnostic procedures. These tests include detection of the virus in cultured fibroblasts exposed to the tested sample, demonstration of circulating antibodies to the virus and detection of the virus in urine or blood by using polymerase chain reaction (PCR). The tissue culture procedures required for detecting the virus are time-consuming and expensive and are considered by some to be less sensitive. Serologic tests for the detection of CMV antibodies rely on the immunologic status of the individual and can vary significantly with time (7).

CMV is known for its wide distribution in humans and a propensity for latency and reactivation during the lifetime of the host. During latency CMV remains latent in the cells, such as leukocytes (8), renal tissues (9) and female genital organ (10).

PCR is the most sensitive procedure and it can detect virus that is either non-infectious, remnant, nonviable, or latent (11). Detection of CMV DNA by PCR in peripheral blood leukocyte is one of the very sensitive markers of CMV disease (7). Because circulating lymphocytes are sites for CMV during latency period, detection of CMV DNA by PCR in plasma should better reflect the status of viremia or active virus infectivity in individuals. Beside more direct measurement of active CMV infection, PCR of plasma, as opposed to PCR of leukocytes, have the advantage of being an easier substrate to process (12).

This study also demonstrated that PCR CMV is more sensitive than CMV-IgM in detecting active CMV disease, as shown in two cases who had evidence of CMV pneumonitis and a positive PCR CMV test but a negative CMV-IgM test. The other two cases who had positive PCR CMV and positive CMV-IgM and did not have evidence of CMV infection in lung tissue may have other

Table 2. Histopathology of lung tissue and CMV markers.

Case No.	Age (mo.)	PCR (plasma) CMV	CMV IgM	CMV IgG	Histopathology of lung tissue
9	2	pos	neg	200	CMV
2	2	pos	neg	200	CMV, Pc*
4	3	pos	>320	200	Pc
8	2	pos	100	200	Pc, atypical pneumocytes*
12	3	neg	neg	neg	Pc
15	4	neg	neg	100	atypical pneumocytes

*Pc : *Pneumocystis carinii*

** atypical pneumocytes : not characteristic of CMV

active sites of CMV infection such as retinitis or colitis.

Interactions between HIV and CMV may occur bidirectionally. Immunosuppression caused by HIV clearly pre-expose the patient to CMV infection. Clinically severe CMV disease is often associated with advanced immunocompromised states. Whether CMV co-infection adversely influences the course of HIV disease is unknown.

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