Cytomegalovirus Co-Infection in Patients with Human Immunodeficiency Virus in Iran

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Abstract- Serum samples from 201 HIV positive patients were collected to determine the seroprevalence of CMV infection in Iranian HIV infected patients during March 2004 until March 2005 using conventional ELISA kits. An antibody level of >1.1 Iu/ml was considered positive. The seroprevalence of CMV infection was 94%.The maximum prevalence of CMV antibody was seen in patients with unsafe sex and IDUs. Prevalence of CMV was much higher in patients with low socioeconomic status and low level of education. 83% of patients with CD4<100 were CMV seropositive. Our study showed that a significantly high prevalence of CMV in HIV positive patients in Iran. By increasing the level of education and socioeconomic status the prevalence of CMV infection decreased.

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Introduction

Cytomegalovirus (CMV) is a ubiquitous organism that can cause infection at any time during the course of life. In various parts of the world, the prevalence of CMV ranges from 40-100% (1).

CMV, a beta-herpes virus, is the major cause of non-Epstein-Barr virus infectious mononucleosis in the general population and an important pathogen in immunocompromised hosts, including patients with AIDS, neonates, and transplant recipients. The risk of exposure to CMV increases with age. As with other herpes viruses, CMV remains latent in the infected host throughout life and rarely reactivates to cause clinical illness except in immunocompromised individuals (2). CMV infection is more prevalent in populations at risk for HIV infection; approximately 75% of injection drug users and >90% of homosexual men who are infected with HIV have detectable IgG antibodies to CMV (3).

The exact sites that allow latent infection to persist are unclear, but polymorph nuclear cells, dendritic cells, endothelial vascular tissue and salivary glands may contain the virus (4,5,6).CMV infection is more prevalent in populations at risk for HIV infection; approximately 75% of injection drug users (IDU) and >90% of homosexual men who are infected with HIV have detectable IgG antibodies to CMV. Higher prevalence rates among homosexual men correlates with the increased risk of exposure associated with receptive anal intercourse (7). In addition, high prevalence rates of CMV IgM antibody in longstanding CMV-seropositive homosexual men suggest that this group is frequently re-exposed to (and at least sometimes reinfected with) differing exogenous strains of CMV (8).

CMV infection is ubiquitous in Iran (9). Previous study determined CMV-IgG positivity in 98% and 100% of women less than 20 and over 40 years old in Tehran (10). After CMV infection in immunocompetent individuals, disease occurs in a small number of cases and it is usually manifested as an infectious mononucleosis like syndrome. It is different from what happens with immunodeficient patients where different clinical manifestations are associated to CMV disease such as retinitis, colitis and encephalitis (11, 12).

These clinical manifestations occurred in 25-40% of AIDS patients before the starting highly active anti-retroviral therapy (HAART) (11, 12). The incidence of CMV disease declined rapidly and significantly by using HAART (13).

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In these patients the disease frequently occurred as a reactivation of latent virus in CMV positive patients. Likewise it has been shown that CD4 levels less than 50 cells/mm^3 are important in the prognosis of clinical manifestation of CMV (14).

A diagnosis of CMV disease can be based on clinical evaluation (e.g., CMV retinitis) but often requires tissue biopsy with histological evidence of viral inclusions and inflammation (e.g., CMV colitis). Culture of CMV from blood, urine, or even biopsied tissue may only reflect infection rather than true end-organ disease. Detection of CMV inclusions, antigens, or nucleic acids in situ is preferred method for making a diagnosis of CMV end-organ disease (15).

The purpose of this prospective study was to assess the prevalence of primary infection with CMV in a population of HIV positive patients in Tehran, Iran using anti-CMV IgG, IgM antibodies –ELISA.

Materials and Methods

A retrospective study of HIV positive patients who were admitted in infectious diseases ward of Imam Khomeini hospital referred from different geographic areas was carried out in Tehran, Iran between March 2004 and March 2005. 201 HIV/AIDS patients were admitted after consenting agreement. Clinical and laboratory data were obtained from patients' records. This research was approved by department of infectious disease at Tehran University of Medical Sciences. Two ml blood was drawn from HIV positive patients on day of admission, and blood samples were centrifuged and transferred to research laboratory. The serum was restored in -20$^{\circ C}$ until the serologic examination (anti-CMV IgG and IgM) were performed on them.

Serologic tests

The evaluation of anti-CMV antibodies were carried out with commercial kits (clone system EIA gen CMV IgG and IgM, biochem immunosystem, Italy S.P.A).

Both kits were used and the results were interpreted as suggested by manufacturer. The sample was considered positive if the antibody titer was more than>1.1 Iu/ml, doubtful if the antibody titer was between 0.9-1.1, and negative if the antibody titer was<0.9. All doubtful test results were repeated.

Statistical analysis

The data were analyzed using the statistical software SPSS (SPSS Inc, Chicago .USA). Statistical analysis was estimated using either chi-square test or Fischer exact test where appropriate. *P* value<0.05 was regarded as statistically significant.

Results

We retrospectively reviewed 201 HIV/AIDS patients, who attended to infectious diseases ward of Imam Khomeini Hospital between March 2004 and March 2005. The age range of patients varied from 3 to 62 years with the mean age of 36.5 years (SD=9.96, SE=0.7) and the mean CD4 count was 339 cells/µl (SD=244.4, SE=17.3). In term of sex, out of 201 patients, 172 were male and 29 were female. The seroprevalence of Anti-CMV IgG Ab was 94% in males and 93% in females. Anti- CMV IgM was negative in all cases. These patients had different level of education and socioeconomic status. 135 patients (67.2%) had elementary education, 52 (25.9%) had high school education and 14 (6.9%) had university education. The prevalence of CMV infection was 96% in patients with elementary education, 90% in high school education, and 85% in university education (*P*=0.09). As is shown in Table 1 by increasing the level of education, prevalence of CMV decreased.

We also evaluated the study results by place of residence in Tehran (North, Central, East, South, and West). From 189 CMV positive patients, 8 (4%) were living in North, 21 (11%) in East, 34 (18%) in West, 49 (26%) in Center, and 77 (41%) in South of Tehran (Table 2).

From 201 patients, HIV transmission modes were including: 67 patients (33.3%) by intravenous drug use, followed by 15 (7.5%) by unsafe sex, 8 (4%) by blood transfusion and 25 (12.5%) HIV patients spouse (patients who had sexual contact with a monogamous spouse infected with HIV), and 14 (7%) by other routes like tattoo, and vertical transmission, 72 (35.7%) had more than one risk factor. Prevalence of CMV infection was 100% in IDU, patients with unsafe sex and blood transfusion, 92.9% in HIV patients spouse and 96.7% in patients with more than one risk factor, but in patients with other routes of infection (tattoo, and vertical transmission) the prevalence of CMV was 72.7% (*P*=0.01). Mean age of HIV positive patients with CMV infection was 36.5 years (SD=9.1, SE=0.64) with a range of 3-58 years. The maximum prevalence of CMV infection was seen in 50-60 years age group (100%). The minimum prevalence of CMV infection was seen in 0-10 years age group (50%).

The prevalence of CMV infection increased gradually by age (*P*=0.001; Table 3).

Table 1. Prevalence of CMV in HIV Positive Patients by Sex and Level of Education

	CMV positive Number (%)	CMV negative Number (%)	*P value*
Sex			
Male	162 (94%)	10 (6%)	Not significant
Female	27 (93%)	2 (7%)	
Education			
Elementary	130 (96.2%)	5 (3.8%)	
High School	47 (90.3%)	5 (9.7%)	0.09
University	12 (85.7%)	2 (14.3%)	

Table 2. The Number (Percent) of HIV Patients with Positive CMV Serology by Site of Living in Tehran

Site of Living in Tehran	Number	Percent
North	8	4%
East	21	11%
West	34	18%
Center	49	26%
South	77	41%
Total	189	100%

We categorized HIV patients by CD4 level into 4 groups: CD4<100, 100<CD4<200, 200<CD4<500 and CD4>500. CMV infection was detected in 83.3% of HIV/AIDS patients with CD4 count<100, in 100% with 100<CD4<200, in 96% with 200<CD4<500, and in 92.9% of those with CD4>500.

As is shown, prevalence of CMV infection in HIV positive patients was similar in different levels of CD4 groups and there was not significant association between prevalence of CMV and CD4 levels (*P*=NS).

Discussion

In most developed countries, human CMV seroprevalence steadily increases after infancy. About 10-20% of children are infected before puberty, while in adults the prevalence of antibodies ranges from 40-100%. Although CMV has a worldwide distribution, infection with CMV is more common in developing countries and in areas of low socioeconomic conditions which is predominantly related to the close contacts within these populations (5, 16).

This high prevalence of CMV infection in the adult population is probably also observed in the HIV infected population. In France, seroprevalence of CMV varies from 70% in the general population to more than 90% in HIV infected patients (17). There are also different data from USA and prevalence of CMV varies from 40-100% in different locations (18, 19).

In our study the seroprevalence of CMV in HIV positive patients was 94% and also there was no association between CMV infection rate and gender (*P*=NS).

From a previous study done in Iran, 98% of women less than 20 years and 100% of those over 40 years of age in Tehran were CMV-IgG positive (mean=99.1%)(20).So in Iran, like other developing countries such as Brazil, and Egypt, CMV is a prevalent infection (21).

All of the cases in our study were CMV-IgG positive and none of them were CMV-IgM positive. Negative results of CMV-IgM may be due to absence of frequent re-exposed to (and at least sometimes reinfection with) differing exogenous strains of CMV which is mostly seen in homosexual men of other countries, while this is not a common route of HIV transmission in Iran (8). We evaluated the level of education. The prevalence of CMV infection was 96% in people with elementary education, 90% in high school, and 85% in university education (*P*=0.09), by increasing the level of education the prevalence of CMV decreased.

Table 3. Prevalence of CMV in HIV Positive Patients by Age Group

Age Group (Year)	0-10	10-20	20-30	30-40	40-50	50-60
CMV Positive, Number (%)	2 (50%)	-	47 (94%)	79 (94%)	52 (96.2%)	9 (100%)
CMV Negative, Number (%)	2 (50%)	-	3 (6%)	5 (6%)	2 (3.8%)	0
Total	4	-	50	84	54	9

We divided the CMV patients into 5 groups by places of residence (South, Central, West, East, and North). In our study most patients were living in South of Tehran (41%) whereas a smaller numbers of patients were living in North of Tehran (4%). Most of population in South of Tehran have lower socioeconomic status comparing to the other places (crowded families, poor hygiene and low income), whereas the socioeconomic level of residents in North of Tehran is higher, so this may be an explain that why CMV infection is more prevalent in patients residing in South of Tehran .This result again is concurrent with previous studies (5,9,19,22).

In USA, CMV is often transmitted sexually (in late adolescence and young adulthood) and asymptomatic viral carriage in semen or cervical secretion is common. CMV antibodies are present at detectable levels in nearly 100% of female sex workers and sexually active homosexual men (19). In other researches there is also a significant difference between the prevalence of CMV in different HIV transmission risk factors. In England and France the maximum prevalence of CMV was seen in homosexual men (22). In our study, the prevalence of CMV in persons with unsafe sex, IDU and blood transfusion was as high as 100%. The minimum prevalence of CMV was seen in patients with other routes of HIV transmission like tattoo, and vertical transmission (72.7%) (P=0.001).

Mean age of CMV positive patients was 36.5 years (SD=9.1, SE=0.64) with the range of 3-58 years. The maximum prevalence of CMV infection was seen in 50-60 years age group (100%) and the minimum prevalence in 0-10 years age group (50%). Therefore, it seems that the prevalence of CMV increases gradually by age. In USA, 1-2% of newborns are infected with CMV (23). Until puberty 10-40% of children are infected with CMV and 50-90% of adult population is seropositive for CMV. There are three peak ages for acquisition of infection, infancy, early childhood and early adulthood (24).

CMV is recognized as an important pathogen in patients with advanced HIV infection in whom it often causes retinitis or disseminated disease, particularly when peripheral CD4 cell counts fall below 50 to 100 10^6/ml (25).

By using HAART therapy the incidence of CMV disease declined rapidly and significantly (13).

Our study evaluated the seroprevalence of CMV in different CD4 count cells. The minimum prevalence of CMV infection was in CD4<100. In other CD4 level groups the prevalence of CMV infection was approximately similar and there was no association between seroprevalence of CMV and CD4 count (P=NS).

Our study showed a significantly high prevalence of CMV infection in HIV/AIDS patients in Iran with the age, level of education, socioeconomic status and CD4 count levels as the main factors affecting the prevalence of CMV infection in these patients.

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