Original Article

Sero-prevalence of *Cytomegalovirus* and *Toxoplasma* Infections among Newly Diagnosed HIV Patients in Iran; Assessing the Correlation with $CD4^+$ Cell Counts

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ABSTRACT

Background and Objective: **Opportunistic infections are the leading cause of death among patients subjected to the human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS). The aim of this study was to compare the seroprevalence of *Cytomegalovirus* (CMV) and toxoplasmosis infection in newly diagnosed HIV infected patients with healthy controls and it's correlation with $CD4^+$ cell counts ($CD4^+$).**

Materials and Methods: **A case controlled study was carried out to investigate CMV and toxoplasmosis serology among newly diagnosed HIV infected patients referred to University affiliated hospital in Tehran and compared them to healthy subjects as control. A total of 100 HIV positive patients and 100 healthy controls were recruited. Clinical characteristics and $CD4^+$ cell counts were evaluated. The statistical package SPSS 17 for windows was used for analysis.**

Results: **Patients with HIV infection had a significantly higher positive serology for CMV than healthy controls (100% vs. 93% $p<0.05$). There was no significant difference between HIV positive and HIV negative patients in terms of toxoplasmosis serology. There was no significant difference between males and females with respect to CMV or toxoplasmosis serology.**

Conclusion: **CMV and toxoplasmosis infection are highly prevalent among HIV infected patients and also healthy controls, with a higher seropositive rate of CMV in HIV cases.**

Key words: **HIV; *Cytomegalovirus*; Toxoplasmosis; Serology**

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Introduction

The prevalence of opportunistic infection in patients with acquired immune deficiency syndrome (AIDS) is approximately 300 times more than aged-matched reference population (1). Defective cell- mediated immunity in human immune deficiency virus (HIV) infected subjects makes them susceptible to reactivation of latent micro organisms and even lead to reactivation of such an opportunistic infections (OIs) which not only impose a varying range of morbidities, but it also causes a high cost for affected individuals and related society (2). There are evidences showing that opportunistic infections (OIs) are more prevalent among HIV seropositive subjects (3).

Cytomegalovirus (CMV) and toxoplasmosis are of these opportunistic pathogens with particular importance in HIV/AIDS patients (4, 5). Many complications including disseminated toxoplasmosis infection (6), blindness, and CMV pseudo tumor have been reported by reactivation of these organisms in immune compromised individuals (7, 8). Besides, there is no vaccine available for CMV and toxoplasmosis yet (9, 10).

Usually decrease in $CD4^+$ cell counts leads to reactivation of these infections. Postmortem studies have shown that CMV viral load is higher than HIV load and is suspected to be the cause of death in most of patients already diagnosed as AIDS (9-12). It is a significant risk for morbidity and mortality in HIV- CMV co- infected patients. Besides, toxoplasmosis encephalitis is the most important cause of cerebral mass lesion in patients infected with HIV (11, 12). Decreased number of $CD4^+$ cells is the culprit mechanism leading to toxoplasmosis encephalitis (13). It is known that highly active antiretroviral therapy treatment (HAART) results in a dramatic decline of CMV infection and a significant survival of HIV patients (14, 15), when cotrimoxazole chemoprophylaxis is considered as an effective strategy in prevention of toxoplasmosis reactivation (16). However, timely diagnosis, early starting of prophylaxis and sufficient treatment are the only available options for these opportunistic infections so far (1, 3).

To our knowledge, there is no evidence supporting higher prevalence of CMV infection among Iranian patients; neither there is recommendation which necessitates early and sharp initiation of prophylaxis for these infections.

The aim of this study is to compare the seroprevalence of CMV and toxoplasmosis infection in newly diagnosed HIV infected patients with healthy controls and to investigate its correlation with $CD4^+$ cell counts.

Materials and Methods

We performed a case controlled study to assess serology of CMV and toxoplasmosis in HIV infected patients attending to the clinic of high risk behavior in a university hospital, Tehran, Iran and compared them to the healthy controls. All participants filled a written informed consent before entering the study.

We follow the national AIDS control organization recommendation (NACO 2007) for diagnosis of HIV infection. Anti HIV antibody was assessed based on ELISA and western blot tests. All the patients were diagnosed within a month from the time of infection and were not on HAART. A total of 100 HIV positive patients and 100 healthy age-matched controls were recruited.

Age under 15 years old, pregnancy, and any type of malignant disorders were considered as exclusion criteria. Demographic data including age, sex were obtained for all the participants. $CD4^+$ cell counts were measured in HIV infected patients. A self completed questionnaire was filled by the patients to assess the mechanism of infection in all HIV subjects. Data were entered the data bases.

The study was carried out according to the principles of declaration of Helsinki. The local Ethics Committee of Tehran University of Medical Sciences approved the study protocols.

Blood samples

Anti HIV antibody was diagnosed by ELISA (IBL kit, Germany) in all the cases and then confirmed by western blot. The diagnosis of CMV was made based on the high or rising titers of anti CMV smooth lip polysaccharide (IgM) antibodies in the serum (IBL kit, Germany). Positive results were confirmed by ELISA (IBL kit, Germany). The diagnosis of toxoplasmosis was also made by high or rising titers of antibodies (IgG) against toxoplasmosis smooth lip polysaccharide in the serum (IBL kit, Germany). Positive results were also confirmed by ELISA (IBL kit, Germany).

$CD4^+$ cell counts were calculated using single platform flowcytometry (Partec-Pas, Germany).

Statistical analysis

Variables distributed normally are presented as mean and standard error of mean (SE M). To compare the prevalence of CMV and toxoplasmosis infections between males and females, and also between HIV^+ and HIV – patients, chi square test was employed.

The statistical package SPSS 17 for windows (Chicago, Illinois, USA), was used for analysis. Differences were considered significant at a *P*- value of lower than 0.05.

Results

All patients were known cases of HIV infection (diagnosed concordantly by ELISA and western blotting), 80% of them were infected by intravenous drug abuse followed by sexual contact (15%) and unknown routes (5%). The most common presentation of them was dermatologic complaints like reactivation of herpes simplex, herpes zoster, seborrheaic dermatitis, and acne which were resistance to treatment. There were 3 patients with toxoplasmosis encephalitis and 4 patients with CMV reactivation.

Mean $CD4^+$ level was 297.65 ± 14.68 cells/µl in HIV infected patients. Nearly 50% of patients presented timely with $CD4^+$ cell counts > 350 cells/µl and none of them had toxoplasmosis or CMV infection at the time of presentation. Primary and clinical characteristics of participants are summarized in Table1.

Table 1- Characteristic data, *cytomegalovirus* and toxoplasmosis seroprevalance rates among Human Immunodeficiency Virus (HIV) infected patients and control group

	HIV Negative (n=100)	**HIV Positive (n=100)**	***P*- Value**
Age (yr)	34.8 ± 0.71	36.04 ± 1.04	>0.05
Males (N, %)	55 (55.0)	78(78)	0.001
***Cytomegalovirus* infection (N, %)**			0.007
Positive Serology	93 (93.0)	100 (100.0)	
Negative Serology	7 (07.0)	0(0.0)	
Toxoplasmosis infection (N, %)			>0.05
Positive Serology	62 (62.0)	65 (65.0)	
Negative Serology	38 (38.0)	35(35.0)	

Variables are expressed as mean ± standard error, unless otherwise is stated

Patients with HIV infection had significantly higher positive serology for CMV than healthy controls (100% vs. 93% $P< 0.05$). The difference between HIV positive and HIV negative patients with respect to toxoplasmosis serology (65% v 62%) was non-significant. There was no significant difference between males and females with respect to CMV or toxoplasmosis serology. There was a significant correlation between age and toxoplasmosis seropositivity in HIV infected patients (P=0.015). Older patients were more susceptible to have positive toxoplasmosis serology.

Discussion

Seroprevalance of different HIV co infections may vary depending geographic region and ethnicity. In the present study, we assess the seroprevalance of IgM CMV and IgG anti *Toxoplasma* antibodies among Iranian HIV infected patients compared with healthy controls. All patients with toxoplasmosis and CMV reactivation had $CD4^+$ cell counts < 350 cells/µl. The seroprevalance of IgM CMV antibody found 100% vs. 93% in HIV infected patients and control group, respectively; as were 65% vs. 62% for IgG anti *Toxoplasma* antibody.

In our study, males constitute the predominant gender; HIV negative group had a grater age compared to HIV positive patients. The main route of transmission was intravenous drug abuse followed by sexual contact, however no significant difference was observed in seroprevalance of above antibodies between patients with different HIV transition mode.

According to CDC (2010) CMV IgM is not solely indicative of primary infection. It also detectable in cases of recent re exposure or reactivation of CMV infection in whom acquired CMV in the past (17).

Despite the effective adventure of HAART which caused a sharp decline in CMV disease in HIV positive patients (18), there is still risk related to HIV seropositivity for co infection of CMV (19, 20). Our finding clearly demonstrated that patients with HIV infection are at increased risk of CMV infection compared to normal controls. However, we did not find any differences between prevalence of toxoplasmosis infection in HIV positive patients and controls. Although these findings do not help to determine whether CMV infection is a link to a higher susceptibility of patients to HIV infection or vice versa, they support the hypothesis that CMV infection play an important role in the pathogenesis of AIDS in HIV infected patients.

There are controversies on the CMV prevalence among HIV infected patients. In a cross sectional study conducted in Ghana, the seroprevalence of CMV infection was 77.6% in HIV seronegative patients while HIV seropositive patients had a prevalence of 59.9%; however it was not statistically significant (21).

In Russia, the seroprevelance of CMV infection was 48.4%, significantly higher in HIV infected patients compared with normal controls (22). Other investigation on Cambodian patients showed the prevalence of CMV infection about 55.8% among HIV infected patients; however, the study did not include any control group to compare the patients with (23). These findings are in the same line with our observation. We also found a higher prevalence of CMV infection in Iranian HIV infected patients in comparison with normal controls. On the other hand the prevalence of CMV infection was 100% in our patients which emphasize the fact that these patients are at the greatest risk for CMV reactivation.

There is a controversy over the beneficial of CMV prophylaxis in HIV seropositive patients,

and most studies state that CMV prophylaxis with ganciclovir should only be considered for patients with $CD4^+$ lymphocyte counts below 50 cells/μL who may be at increased risk for retinitis. However Combination antiretriviral therapy (ART) alone has reduced the rate of CMV in HIV infected patients by 75% (24, 25).

Toxoplasmosis is an opportunistic protozoon prevalent all over the world and one of the most important opportunistic infections in HIV infected patients.

Anti-*Toxoplasma* IgG can be estimated as a screening test to detect latent infection in all HIV infected patients (26-30). IgM anti *Toxoplasma* antibody seems to have no additional diagnostic or therapeutic benefit in these patients (28-32).

Different studies around the world show a wide spectrum of *T. gondi* seropositivity as 3-42% in USA (26), 74.5 in south brazil (31), 67.3% in Paris (30), 67.8% in India (28), 30% in Czech Republic (27) and 12.5% in China (33). Our findings complement those of previous studies of patients with HIV infection as well as normal population regarding toxoplasmosis infection. Toxoplasmosis seropositivity rates in Iranian population are estimated as the range of 41 -68 % in different provinces, furthermore studies conducted on HIV infected patients showed a higher rates of seropositivity (49.7% – 77.4%) in these patients (32, 34-36). As stated, in our study this rate estimated 65%. This difference could be related to recruited subjects, using different assay and the year of study (8, 37-38).

In a cohort study conducted in Czech Republic on 626 HIV positive patients for 18 years, most HIV seropositive patients (98.1%) were showed to be infected before toxoplasmosis infection (27). In an another case-control study conducted in Ethiopia, the incidence of toxoplasmosis infection was 93% and 86.7% in HIV infected and HIV uninfected individuals, respectively. They also found a relationship between toxoplasmosis infection and age which is in consistent with our findings (39). However in a case control study conducted in India, the seroprevalence of toxoplasmosis infection was 34% in immune competent host, and 67.8% in HIV positive patients which was significantly higher. They also showed higher incidence of toxoplasmosis seropositivity in older patients (28).

In addition, compared to the other countries, the positive toxoplasmosis serology is relatively higher in Iran (32, 35, 38, 39). The differences between seroprevalence rates in different areas may due to the climate condition, nutritional behavior and possessing cats.

Furthermore some Studies show a significant decrease in *T. gondi* seropositivity as the educational level increased (28). Hand hygiene and consumption of well cooked meats could also affect *T. gondi* seropositivity. Studies suggest primary prophylaxis against toxoplasmosis in *T. gondi*i-seropositive (IgG) patients with $CD4^+$ <100 cells/μL without considering clinical status, and in patients with opportunistic infection or malignancy in $CD4^+$ <200 cells/μL (25).

This study showed that toxoplasmosis and *cytomegalovirus* are of high incidence in HIV positive patients and given the life-threatening consequences of these infections, the patients – specifically those with lower $CD4^+$ cell counts – are strongly recommended to be monitored regularly. It is suggested that multicenter studies with more samples be carried out on these patients while taking into account their medications.

The main limitation of the present study is its cross sectional feature which preclude the determination of the direction of causality. However, a relatively larger sample size in comparison to the other studies may be vanished such a concern.

Conclusion

We showed that CMV infection has a higher prevalence among HIV infected patients while there was no difference in toxoplasmosis serology among HIV infected patients and controls. This may help for a better management of HIV infection in developing countries like Iran which are endemic for both infections.

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References

1. Saha K, Firdaus R, Santra P, Pal J, Roy A, Bhattacharya MK, *et al*. Recent pattern of Co infection amongst HIV seropositive individuals in tertiary care hospital, Kolkata. Virol J 2011;8:116.

2. Meyer CN, Skinhoj P, Prag J. Bacteremia in HIV-positive and AIDS patients: Incidence, species distribution, risk-factors. Outcome, and influence of long-term prophylactic antibiotic treatment. Scand J Infect Dis 1994;26(6):635-42.

3. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR Recomm Rep 1992;41(RR-17):1-19.

4. Griffiths PD. CMV as a cofactor enhancing progression of AIDS. J Clin Virol 2006;35(4):489-92.

5. Okome-Nkoumou M, Boguikouma JB, Kombila M. Opportunistic diseases in HIV-infected patients at the Jeanne Ebori Foundation in Libreville, Gabon . Med Trop (Mars) 2006;66(2):167-71.

6. Ouermi D, Simpore J, Belem AM, Sanou DS, Karou DS, Ilboudo D, *et al*. Co-infection of *Toxoplasma* gondii with HBV in HIV-infected and uninfected pregnant women in Burkina Faso. Pak J Biol Sci 2009;12(17):1188-93.

7. Kedhar SR, Jabs DA. *Cytomegalovirus* retinitis in the era of highly active antiretroviral therapy. Herpes 2007;14(3):66-71.

8. Kelesidis T, Tozzi S, Mitty R, Worthington M, Fleisher J. *Cytomegalovirus* pseudotumor of the duodenum in a patient with AIDS: an unrecognized and potentially treatable clinical entity. Int J Infect Dis 2009; 14(4): 274-82.

9. Fuse S, Molloy MJ, Usherwood EJ. Immune responses against persistent viral infections: Possible avenues for immunotherapeutic interventions. Crit Rev Immunol 2008;28(2):159-83.

10. Chakravarti A, Kashyap B, Matlani M. *Cytomegalovirus* infection: An Indian perspective. Indian J Med Microbiol 2009;27(1):3-11.

11. Alfonso Y, Fraga J, Fonseca C, Jimenez N, Pinillos T, Dorta-Contreras AJ, *et al*. Molecular diagnosis of *Toxoplasma gondii* infection in cerebrospinal fluid from AIDS patients. Cerebrospinal Fluid Res 2009;6:2.

12. Murata M, Furusyo N, Otaguro S, Nabeshima S, Ariyama I, Hayashi J. HIV infection with concomitant cerebral toxoplasmosis and disseminated histoplasmosis in a 45-year-old man. J Infect Chemother 2007;13(1):51-5.

13. Nissapatorn V. Lessons learned about opportunistic infections in southeast Asia. Southeast Asian J Trop Med Public Health 2008;39(4):625-41.

14. Gona P, Van Dyke RB, Williams PL, Dankner WM, Chernoff MC, Nachman SA, *et al*. Incidence of opportunistic and other infections in HIV-infected children in the HAART era. JAMA 2006;296(3):292-300.

15. Verbraak FD, Boom R, Wertheim-van Dillen PM, van den Horn GJ, Kijlstra A, de Smet MD. Influence of highly active antiretroviral therapy on the development of CMV disease in HIV positive patients at high risk for CMV disease. Br J Ophthalmol 1999;83(10):1186-9.

16. Leport C, Derouin F, Morlat P, Chene G, Vilde JL. Toxoplasmosis in immunosuppressed patients. Contribution to the knowledge of toxoplasmic infection. Med Mal Infect 1996;3:437-40.

17. Centers for Disease Control and Prevention. (2010). CMV. Retrieved from http://www.cdc.gov/cmv/clinical/

lab-tests.html.
18. Mocroft A, Sabin CA, Youle M, Madge S, Tyrer M, Devereux H, *et al*. Changes in AIDS-defining illnesses in a London Clinic, 1987-1998. J Acquir Immune Defic Syndr 1999;21(5):401-7.
19. Salmon-Ceron D, Mazeron MC, Chaput S, Boukli N, Senechal B, Houhou N, *et al*. Plasma *cytomegalovirus* DNA, pp65 antigenaemia and a low CD4 cell count remain risk factors for *cytomegalovirus* disease in patients receiving highly active antiretroviral therapy. AIDS 2000;14(8):1041-9.
20. Deayton JR, Prof Sabin CA, Johnson MA, Emery VC, Wilson P, Griffiths PD. Importance of *cytomegalovirus* viraemia in risk of disease progression and death in HIV-infected patients receiving highly active antiretroviral therapy. Lancet 2004;363(9427):2116-21.
21. Adjei AA, Armah HB, Gbagbo F, Boamah I, Adu-Gyamfi C, Asare I. Seroprevalence of HHV-8, CMV, and EBV among the general population in Ghana, West Africa. BMC Infectious Diseases 2008;8.
22. Kalugina MI, Orlova OA, Rybalkina TN, Karazhas NV. The rate of human herpes virus type 6 and *cytomegalovirus* among HIV-infected and healthy women from Chelyabinsk and Moscow. Zhurnal mikrobiologii, epidemiologii, i immunobiologii 2007(3):81-3.
23. Micol R, Buchy P, Guerrier G, Duong V, Ferradini L, Dousset JP, *et al*. Prevalence, risk factors, and impact on outcome of *cytomegalovirus* replication in serum of Cambodian HIV-infected patients (2004-2007). Journal of Acquired Immune Deficiency Syndromes 2009;51(4):486-91.
24. Spector SA, McKinley GF, Lalezari JP, Samo T, Andruczk R, Follansbee S, *et al*. Oral ganciclovir for the prevention of *cytomegalovirus* disease in persons with AIDS. Roche Cooperative Oral Ganciclovir Study Group. N Engl J Med 1996;334(23):1491-7.
25. Centers for Disease Control and Prevention. (2002). Guidelines for Preventing Opportunistic Infections Among HIV-Infected Persons. Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5108a1.htm
26. Falusi O, French AL, Seaberg EC, Tien PC, Watts DH, Minkoff H, *et al*. Prevalence and predictors of *Toxoplasma* seropositivity in women with and at risk for human immunodeficiency virus infection. Clin Infect Dis 2002;35(11):1414-7.
27. Kodym P, Hrda S, Machala L, Rozsypal H, Stankova M, Maly M. Prevalence and incidence of *Toxoplasma* infection in HIV-positive patients in the Czech Republic. J Eukaryot Microbiol 2006;53 Suppl 1:S160-1.
28. Meisheri YV, Mehta S, Patel U. A prospective study of seroprevalence of toxoplasmosis in general population, and in HIV/AIDS patients in Bombay, India. J Postgrad Med 1997;43(4):93-7.
29. NCCLS. Clinical Use and Intrepretation of Serologic Tests for *Toxoplasma gondii*; Approved Guideline. NCCLS document M36-A [ISBN 1-56238-523-2]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2004.
30. Jeannel D, Niel G, Costagliola D, Danis M, Traore BM, Gentilini M. Epidemiology of toxoplasmosis among pregnant women in the Paris area. Int J Epidemiol 1988 ; 17(3):595-602.
31. Spalding SM, Amendoeira MR, Klein CH, Ribeiro LC. Serological screening and toxoplasmosis exposure factors among pregnant women in South of Brazil. Rev Soc Bras Med Trop 2005; 38:173–7.
32. Salahi-Moghaddam A, Hafizi A. A serological study on *Toxoplasma* gondii infection among people in south of Tehran, Iran. Korean J Parasitol 2009;47(1):61-3.
33. Xiao Y, Yin J, Jiang N, Xiang M, Hao L, Lu H, *et al*. Seroepidemiology of human *Toxoplasma* gondii infection in China. BMC Infect Dis 2010; 10:4.
34. Daryani A, Sharif M, Meigouni M. Seroprevalence of IgG and IgM anti-*Toxoplasma* antibodies in HIV/AIDS patients, northern Iran. Asian Pac J Trop Med 2011; 4(4):271-4.
35. Fallah M, Rabiee S, Matini M, Taherkhani H. Seroepidemiology of toxoplasmosis in primigravida women in Hamadan, Islamic Republic of Iran, 2004. East Mediterr Health J 2008;14(1):163-71.
36. Mohraz M, Mehrkhani F, Jam S, SeyedAlinaghi S, Sabzvari D, Fattahi F, *et al*. Seroprevalence of toxoplasmosis in HIV(+)/AIDS patients in Iran. Acta Med Iran 2011;49(4):213.

37. Mostafavi SN, Ataei B, Nokhodian Z, Yaran M, Babak A.Seroepidemiology of *Toxoplasma* gondii infection in Isfahan province, central Iran: A population based study. J Res Med Sci 2011; 16(4):496-501.

38. Shimelis T, Tebeje M, Tadesse E, Tegbaru B, Terefe A. Sero-prevalence of latent *Toxoplasma gondii* infection among HIV-infected and HIV-uninfected people in Addis Ababa, Ethiopia: A comparative cross-sectional study. BMC Res Notes 2009;2:213.

39. Richards FO Jr, Kovacs JA, Luft BJ. Preventing toxoplasmic encephalitis in persons infected with human immunodeficiency virus. Clin Infect Dis 1995 Suppl (1):49-56.