Toxoplasma gondii and human immunodeficiency virus co-infection in western Iran: A cross sectional study

Naser Nazari1, Arezoo Bozorgomid2, Alireza Janbakhsh3, Farshid Bashiri3

1Department of Medical Parasitology and Mycology, Faculty of Medicine, Kermanshah University of Medical Sciences, Shahid Shiroudi blvd., Daneshgah St, Kermanshah, Iran
2Department of Microbiology, Asadabad School of Medical Sciences, Asadabad, Iran
3Department of Infection Disease, Faculty of Medicine, Kermanshah University of Medical Sciences, Shahid Shiroudi blvd., Daneshgah St, Kermanshah, Iran

ARTICLE INFO

Article history:
Received 22 August 2017
Received in revised form 30 September 2017
Accepted 15 November 2017
Available online 2 January 2018

Keywords:
Seroepidemiology
Toxoplasmosis
HIV
Iran

ABSTRACT

Objective: To determine Toxoplasma gondii (T. gondii) as a cause of morbidity and mortality in human immunodeficiency virus (HIV) infected patients by ELISA method and to investigate toxoplasmosis-associated risk factors. Methods: This cross sectional study was conducted with 385 patients with HIV/AIDS referred to the behavior disease consultation center in Kermanshah, Iran between May 2012 and June 2013. Anti-Toxoplasma IgG and IgM antibodies were measured by using the ELISA method. Also, the relationship between the infection and age, gender, education, occupation, antiretroviral status, CD4+ cell counts and some other factors of the patients were assessed. P<0.05 was considered statistically significant. Results: Among the HIV-positive patients, 40.8% (157/358) and 2.6% (10/358) patients were positive for anti-T. gondii IgG and IgM antibodies, respectively. The mean CD4+ count in the patients with HIV was 327 cells/µL. A significant correlation was observed between the toxoplasmosis infections with the age group (P<0.05). Nevertheless, no statistically significant difference was found between gender, living area, educational level, occupational, marital status, antiretroviral status, prophylaxis, CD4+ cell count and toxoplasmosis. Conclusions: This study revealed that the patients with HIV infection were at the high risk of developing toxoplasmosis disease especially those patients who do not receive antiretroviral therapy and prophylaxis. The development and use of the effectiveness-based guidelines for controlling and the prevention reactivation of the latent T. gondii infection is required.

1. Introduction

Toxoplasma gondii (T. gondii) as a ubiquitous parasite is one of the most common zoonotic parasitic diseases in almost all warm-blooded vertebrates across the world. It is estimated that 1/3 of the human population in the world has been infected by this protozoan parasite[1]. The primary infection with T. gondii can be asymptomatic and self-limiting in immunocompetent individuals[1]. However, in pregnant women, immunocompromised patients such as those with acquired immune deficiency syndrome (AIDS) and patients receiving chemotherapy or immunosuppressive drugs, an infection with T. gondii may be reactivated in various organs especially brain[2].

Cerebral toxoplasmosis is an important health-related complication in patients with AIDS, which is often observed in the advanced stages of the disease and can lead to morbidity and mortality. In

©2018 by the Asian Pacific Journal of Tropical Medicine. All rights reserved.
the absence of prophylaxis strategies, 30%–40% of HIV-infected patients will develop toxoplasmic encephalitis (TE)\(^3\). Suitable prophylaxis can effectively reduce the rate of TE. Therefore, a timely diagnosis and treatment of \(T. gondii\) serious affect the clinical management of patients with HIV. Additionally, with advent of highly active antiretroviral therapy (HAART) and its effect in improving the immunological status of patients with AIDS have reduced the incidence of TE and death associated with it.

In Iran, the first case of the HIV infection was detected in 1986. By 2014, 27,888 Iranians were reported to be infected with HIV among which 12.63% (3,300 individuals) lived in the Kermanshah Province in the west of Iran\(^4\). Despite our knowledge on the epidemiology of the HIV infection in Iran, little information is available about opportunistic infections in the population of HIV patients. A recent systematic review study of toxoplasmosis in Iranian immunocompromised patients including HIV/AIDS, cancer, and transplantation showed that the rate of seroprevalence was 50.01%\(^5\). Due to a lack of information on the toxoplasmosis serologic status among patients with HIV in Kermanshah, Iran, the risk for damage to the brain and the high morbidity rate of these patients, this study aimed to evaluate the seroepidemiology of \(T. gondii\) infection in patients with HIV. Moreover, improving our knowledge about the seroepidemiology of toxoplasmosis among patients with HIV/AIDS can help us design effective strategies for controlling and preventing this disease.

2. Methodology

2.1. Sample collection and epidemiological factors

This cross-sectional study was carried out with 385 patients with HIV/AIDS referred to the behavior disease consultation center (BDCC) in Kermanshah in the West of Iran from May 2012 to June 2013. The study participants were diagnosed with HIV and were undergoing the follow-up process by the healthcare professionals in the BDCC. The demographic and epidemiologic characteristics of the patients were collected through interviews. In addition, the patients were evaluated with regard to the presence of co-infections, receiving anti-\(Toxoplasma\) prophylaxis and antiretroviral therapy (ART). The CD4\(^+\) T-lymphocyte counts for each patient were measured by the flow cytometry procedure and documented in the patient health file. A sample consisting of 5 mL of venous blood was collected from each person. After the separation of serum, it was labeled and stored at 20 °C until the time of use.

2.2. ELISA

The sample was tested for anti-\(Toxoplasma\) IgG and IgM antibodies by using the ELISA technique according to the manufacturers’ instructions (\(Toxoplasma\) IgG and IgM kit, PISHTAZ TEB\(^\circ\), Tehran, Iran). The optical density of IgG and IgM antibody titers were read at 450 nm. The sample containing above 11 IU/mL was considered positive. The patient was diagnosed negative when the titer the serum IgG and IgM antibodies were lower than 9 IU/mL. Those patients with the range of antibody titers between 9 and 11 IU/mL were considered doubtful positive.

2.3. Ethical clearance

This study was approved by the Research Ethics Committee affiliated with the Kermanshah University of Medical Sciences, Kermanshah, Iran. All the patients were requested to sign the written informed consent form.

2.4. Statistical analysis

Descriptive and inferential statistics via the SPSS software v.16 was used for data analysis. The Chi-squared and Fisher’s exact tests were used to report the associations between variables. \(P<0.05\) was considered statistically significant.

3. Results

In this study, 385 patients consisting of 281 males and 104 females participated. The age range of the patients was 3–68 years and the mean age of the male and female patients was 37.9 years and 34.0 years, respectively. Also, 40.8% (157/385) and 2.6% (10/385) patients were positive for anti-\(T. gondii\) IgG and IgM antibodies, respectively. The prevalence of IgG and IgM antibodies was higher in the age group of 46–60 years, which was statistically significant (\(P=0.01\) and \(P=0.01\), respectively).

No statistically significant association was reported between the seroprevalence of \(Toxoplasma\) infection (seropositivity to IgM or IgG antibodies) and gender, the level of education, occupation, residence, marital status, prophylaxis status and use of azithromycin (\(P>0.05\)) (Table 1).

The ART was received by 203 patients with HIV as follows: zidovudine/lamivudine/nevirapine \((n=24)\), tenofovir/emtricitabine/nevirapine \((n=5)\), zidovudine/lamivudine/efavirenz \((n=162)\) and tenofovir/emtricitabine/lopinavir/ritonavir \((n=12)\).

The majority of the patients had the history of infection with hepatitis C \((n=190)\), hepatitis B \((n=17)\), tuberculosis \((n=69)\) and candidiasis \((n=42)\). About 41.1% \((78/190)\) patients with hepatitis C had \(Toxoplasma\) seropositivity for the anti-\(Toxoplasma\) IgG antibody and 4 patients with hepatitis C had seropositivity for the anti-\(Toxoplasma\) IgM antibody. However, no statistically significant association was reported between seropositivity and the above-mentioned infections \((P>0.05)\).
The mean CD4+ lymphocyte count of the patients with HIV was 327 cells/µL. About 35.8% (28/78) of the patients with HIV who had CD4+ count below 100 cells/µL were seropositive to the Toxoplasma IgG antibody. Regarding chemoprophylaxis in HIV, 14 and 7 Toxoplasma-seropositive patients with a CD4+ cell count < 100 cells/mm³ were not receiving ART and prophylaxis, respectively. No statistically significant association was observed between the patient seropositivity and CD4+ T cell count (P>0.05). The frequency of seropositivity and antiretroviral use related to the levels of CD4+ T-lymphocytes are shown in Table 2.

4. Discussion

Opportunistic infections (OIs) are one of the identified causes for enhancing immunodeficiency in patients with HIV. Also, T. gondii as one of the OIs causes cerebral toxoplasmosis that can be a major threat to the health of patients with HIV. Antiretroviral drugs and HAART have suppressed HIV viral replication and increased CD4+ T-cell counts. Therefore, the incidence of OIs in these patients has reduced[6].

This study aimed to investigate the toxoplasmosis serology in patients with HIV referred to the BDCC in Kermanshah, Iran. In total, 40.8% of the patients were seropositive for the anti-T. gondii
IgG antibody. This seroprevalence did not vary greatly from previous study performed among the general population in the same region (36.3%) [7]. The results of this study revealed that the HIV infection did not increase the risk for toxoplasmosis. A probable reason is that the patients mainly acquired this infection during childhood and adolescence and before the HIV infection. A meta-analysis study among patients with HIV/AIDS in Iran showed that the risk for toxoplasmosis was 50.05% [5]. The lower risk in our study can be attributed to differences in lifestyles, geographic area and weather conditions. Kermanshah province has a moderate and mountainous climate. It is rainy mostly in winters and is moderately warm in summers. Therefore, climate effects influence the survival of oocysts in this environment. However, this infection is widespread in the humid and warm weathers than in other weathers. Furthermore, the consumption of undercooked or raw meat is a risk factor for the infection with toxoplasmosis. A study reported that 18.2% of the sheep cattle and goats in Kermanshah province had the serological evidence for the T. gondii infection [8]. Therefore, patients with HIV are at the risk for acquiring the toxoplasmosis infection in this area.

In this study, the prevalence of the anti-T. gondii IgM antibody was 2.6% (10/385) including eight men and two women. Nevertheless, no statistically significant difference was observed (P>0.05). Regarding previous studies, a study from Ethiopia showed that anti-T. gondii IgM antibodies seroprevalence in HIV seropositive individuals, was 10.7% [9]. However, Rahimi et al. reported that no person had the IgM anti-T. gondii antibodies in Mazandaran Province, North of Iran [10]. Shen et al. found three patients (1.2%) with the anti-Toxoplasma IgM antibody [11].

Among all patients with HIV, 112 men (112/281, 39.9%) and 45 women (45/104, 43.3%) were positive for the anti-T. gondii IgG antibody. Although few studies have reported vertical transmission of T. gondii through reactivation of toxoplasmosis, it is relatively rare [12,13]. No statistically significant difference was detected in the seroprevalence of the anti-Toxoplasma IgG antibody between males and females (P>0.05).

The overall seroprevalence was highest in the 46–60 year age group (51.8%, 28/53) in his study. The study of Zhang et al. has shown the highest prevalence of disease in 3rd and 4th decades of life [14]. Nonetheless, Walle et al. reported the highest prevalence rate in 21–30 year age group [9]. This can probably be explained by the prolonged exposure time with the increase of patient age.

Many studies showed that more Iranian patients with HIV were illicit drug abusers [15,16]. The illicit drug users are at the high risk for the toxoplasmosis infection, particularly due to inferior socio-economical, occupational and nutritional situations [17,18]. In this study, approximately 1/3 of the patients were unemployed. However, no statistically significant difference was observed between occupation, the educational level, marital status and residence areas and seroreactivity to the anti-T. gondii antibodies.

Azithromycin is used for the treatment or as the prophylaxis for Mycobacterium avium, subspecies paratuberculosis, but it can also provokes Crohn’s disease. Since azithromycin can be an alternative treatment for the toxoplasmosis [19], its correlation with seroepidemiology of the infection was assessed. However, no statistically significant difference was reported between the T. gondii seropositivity and azithromycin.

The mean CD4+ lymphocyte count of the patients with HIV was 327 cells/µL while the T. gondii seropositive had the mean CD4+ lymphocyte count of 346 cells/µL. A total of 20.2% (78/385) of the study participants had CD4+ T lymphocyte count less than 100 cells/µL. It could be concluded that the patients with HIV in the Kermanshah region should be considered at the high risk for developing the toxoplasmosis disease, especially when CD4+ T-cells count was below 100 cells/µL.

The ART and cotrimoxazole prophylaxis were not used by 47.3% (182/385) and 55.6% (214/385) of the patients in this study, respectively. Also, HIV-infected patients who did not receive ART and cotrimoxazole prophylaxis had 40% (72/182) and 39% (84/214) anti-Toxoplasma IgG antibodies, respectively, indicating a high risk of the reactivation of dormant cysts. It is noteworthy that 30%–40% of toxoplasmosis patients with AIDS finally developed TE [3]. One HIV-infected patient (3.3%) in this study had the history of TE and two patients had the history of clinical toxoplasmosis. All of them were seropositive to the Toxoplasma infection.

A systematic review and meta-analysis study reported the incidence of OIs and the impact of antiretroviral therapy among 491 608 patients with HIV between 1990 and 2013. Overall, a major reduction in the risk of infection during the first year of treatment using the ART and with the largest effect on oral candidiasis, Pneumocystis pneumonia and toxoplasmosis (range from 57%–91%) [20]. Based on our findings, no statistically significant differences between the T. gondii seropositivity with the antiretroviral treatment and cotrimoxazole prophylaxis were reported.

There was no remarkable difference between the T. gondii seropositivity with the CD4+ lymphocyte count, which is consistent with the reports of studies from Ethiopia and Morocco [21,22]. Another study in Jahrom, Iran found a correlation between the CD4+ count, 100 cells/µL and the Toxoplasma seropositivity [23]. Although CD4+ T cells play a major role in protection against intracellular protozoan parasites such as T. gondii as the producers of IFN-γ, CD8+ T cell and NK cell also are important effector cells in the control of parasite reactivation during HIV infection [24].

As a limitation of this study, the patients were referred to one BDCC that could not reflect the real prevalence of toxoplasmosis among patients with HIV. Therefore, the investigation of associations between the risk factors needs the inclusion of more healthy and infected patients in future studies. Also, approximately 70% of the patients were male and did not allow a perfect gender-specific data analysis. Additionally, monitoring of the antiretroviral treatment and adherence to it and the possibility of adverse reactions were not studied. A study of patients with HIV/AIDS in Brazil showed that the antiretroviral treatment was not used regularly by 32% of patients [25].

The results of this study highlighted the high risk for clinical toxoplasmosis in individuals with HIV, because of the possibility of reactivation of the infection in the seropositive patients who do not adhere to the ART and infection in seronegative patients without primary prophylaxis. TE should be suspected in all people with advanced HIV infection. Undiagnosed patients with HIV may...
present their initial symptoms of their disease with TE.

Further studies are needed to determine the prevalence of TE in patients with HIV. Moreover, there is a need to investigate *T. gondii* strains in patients for evaluating the population genetic structure of *T. gondii* and the association between the parasite genotype and the disease patterns in patients with HIV.

**Conflict of interest statement**

We have no conflict of interest related to this work.

**Acknowledgment**

The authors would like to thank the Behavior Disease Consultation Center (BDCC) in Kermanshah, Iran for their kind assistance during this research project.

**References**

[1] Halonen SK, Weiss LM. Toxoplasmosis. *Handb Clin Neurol* 2013; 114: 125-145.

[2] Montoya J, Liesenfeld O. Toxoplasmosis. *Lancet* 2004; 363: 1965-1976.

[3] Luma HN, Tchaleu BC, Mapoure YN, Temfack E, Doualla MS, Halle MP, et al. *Toxoplasma* encephalitis in HIV/AIDS patients admitted to the Douala general hospital between 2004 and 2009: A cross sectional study. *BMC Res Notes* 2013; 6: 146.

[4] Khademi N, Reshadat S, Zangenhe A, Saeidi S, Ghasemi SR, Rajabi-Gilan N, et al. A comparative study of the spatial distribution of HIV prevalence in the metropolis of Kermanshah, Iran, in 1996-2014 using geographical information systems. *HIV Med* 2016; 18(3): 220-224.

[5] Ahmadpour E, Daryani A, Sharif M, Sarvi S, Aarabi M, Mizani A, et al. Prevalence and associated factors of HIV Infection among male prisoners in Tehran, Iran. *Arch Iran Med* 2017; 20(6): 356-360.

[6] Sharifi H, Mirzazadeh A, Noroozi A, Marshall BD, Farhoudian A, Higgs P, et al. Patterns of HIV risks and related factors among people who inject drugs in Kermanshah, Iran: A latent class analysis. *J Psychoactive Drugs* 2017; 49(1): 69-73.

[7] Razzagh EM, Movaghar AR, Green TC, Khoshnoosh K. Profiles of risk: A qualitative study of injecting drug users in Tehran, Iran. *Harm Reduct J* 2006; 3(1): 1.

[8] Vazirian M, Nassirimanesh B, Zamani S, Ono-Kihara M, Kihara M, Ravari SM, et al. Needle and syringe sharing practices of injecting drug users participating in an outreach HIV prevention program in Tehran, Iran: A cross-sectional study. *Harm Reduct J* 2005; 2(1): 1.

[9] Lashay A, Mirshahi A, Parandini N, Riazi Esfahani H, Mazloumi M, Reza Lashay M, et al. A prospective randomized trial of azithromycin versus trimethoprim/sulfamethoxazole in treatment of toxoplasmic retinochoroiditis. *J Infect Dis* 2013; 262(2): 120-125.

[10] Low A, Gavriilidis G, Larke N, B-Lajoie MR, Drouin O, Stover J, et al. Incidence of opportunistic infections and the impact of antiretroviral therapy among HIV-infected adults in low-and middle-income countries: A systematic review and meta-analysis. *Clin Infect Dis* 2016; 62(12): 1595-1603.

[11] Ngobeni R, Samie A. Prevalence of *Toxoplasma gondii* IgG and IgM and associated risk factors among HIV-positive and HIV-negative patients in Vhembe district of South Africa. *Afri J Infect Dis* 2017; 11(2): 1-9.

[12] Razzagh EM, Movaghar AR, Green TC, Khoshnoosh K. Profiles of risk: A qualitative study of injecting drug users in Tehran, Iran. *Harm Reduct J* 2006; 3(1): 1.

[13] Campos FA, Andrade GM, Lanna Ade P, Lage BF, Assumpção MV, Pinto JA. Incidence of congenital toxoplasmosis among infants born to HIV-coinfected mothers: Case series and literature review. *Braz J Infect Dis* 2014; 18(6): 609-617.

[14] Zhang YB, Cong W, Li ZT, Bi XG, Xian Y, Wang YH, et al. Seroprevalence of *Toxoplasma gondii* infection in patients of intensive care unit in China: A hospital based study. *Biomed Res Int* 2015; 2015: 908217.

[15] Seyed Alinaghi S, Farhoudi B, Mohraz M, Golsoorat Pahlaviani F, Hosseini M, Farnia M, et al. Prevalence and associated factors of HIV Infection among male prisoners in Tehran, Iran. *Arch Iran Med* 2017; 20(6): 356-360.

[16] Sharifi H, Mirzazadeh A, Noroozi A, Marshall BD, Farhoudian A, Higgs P, et al. Patterns of HIV risks and related factors among people who inject drugs in Kermanshah, Iran: A latent class analysis. *J Psychoactive Drugs* 2017; 49(1): 69-73.

[17] Razzagh EM, Movaghar AR, Green TC, Khoshnoosh K. Profiles of risk: A qualitative study of injecting drug users in Tehran, Iran. *Harm Reduct J* 2006; 3(1): 1.

[18] Vazirian M, Nassirimanesh B, Zamani S, Ono-Kihara M, Kihara M, Ravari SM, et al. Needle and syringe sharing practices of injecting drug users participating in an outreach HIV prevention program in Tehran, Iran: A cross-sectional study. *Harm Reduct J* 2005; 2(1): 1.

[19] Lashay A, Mirshahi A, Parandini N, Riazi Esfahani H, Mazloumi M, Reza Lashay M, et al. A prospective randomized trial of azithromycin versus trimethoprim/sulfamethoxazole in treatment of toxoplasmic retinochoroiditis. *J Infect Dis* 2013; 262(2): 120-125.

[20] Low A, Gavriilidis G, Larke N, B-Lajoie MR, Drouin O, Stover J, et al. Incidence of opportunistic infections and the impact of antiretroviral therapy among HIV-infected adults in low-and middle-income countries: A systematic review and meta-analysis. *Clin Infect Dis* 2016; 62(12): 1595-1603.

[21] Ngobeni R, Samie A. Prevalence of *Toxoplasma gondii* IgG and IgM and associated risk factors among HIV-positive and HIV-negative patients in Vhembe district of South Africa. *Afri J Infect Dis* 2017; 11(2): 1-9.

[22] Yohanes T, Debalke S, Zemene H, Amare TB. Prevalence of *Toxoplasma gondii* infection and associated risk factors among HIV-infected individuals at Arba Minch hospital, South Ethiopia. *AIDS Res Treat* 2014; 2014: 652941.

[23] Rehanzadeh H, Sayadi F, Shadmand E, Nasab SD, Yazdi HR,Solhjoo K, et al. Seroprevalence of *Toxoplasma gondii* among HIV patients in Jahrom, Southern Iran. *Korean J Parasitol* 2017; 55(1): 99-103.

[24] Kodym P, Malý M, Beran O, Jilich D, Rozsypal H, Machala L, et al. Incidence, immunological and clinical characteristics of reactivation of latent *Toxoplasma gondii* infection in HIV-infected patients. *Epidemiol Infect* 2015; 143(3): 600-7.

[25] Xavier GA, Cademartori BG, Cunha Filho NA, Farias NA. Evaluation of seroepidemiological toxoplasmosis in HIV/AIDS patients in the south of Brazil. *Rev Inst Med Trop Sao Paulo* 2013; 55(1): 25-30.