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Seroprevalence of IgG and IgM anti-*Toxoplasma* antibodies in HIV/AIDS patients, northern Iran

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ABSTRACT

Objective: To determine the seroprevalence of anti-*Toxoplasma gondii (T. gondii)* IgG and IgM antibodies in HIV/AIDS patients and uninfected subjects. **Methods:** This cross sectional survey was carried out on 78 healthy and 62 HIV$^+$/AIDS individuals in northern Iran between September 2007 and October 2008. Five mL of blood samples were collected from each person in case and control groups. Determination of CD4$^+$ counts was performed by flow cytometry. The serum separated from blood samples was evaluated by conventional ELISA technique to determine the presence of antibodies to *T. gondii*. **Results:** Forty eight out of 62 (77.4%) HIV/AIDS serum samples were found positive for anti-*T. gondii* IgG antibody, compared with 59 among 78 (75.6%) HIV negative samples from the same area (P>0.05). Six out of 62 (9.7%) HIV$^+$/AIDS patients showed anti-*T. gondii* IgM antibody in their serum samples, compared with 7 among 78 (9%) HIV negative samples(P>0.05). The mean of CD4$^+$ counts in HIV$^+$/AIDS was (430.8±182.3) cells/ μ L and in control group was (871.0±243.3)% cells/ μ L (P<0.01). CD4$^+$ estimation in 5 (11.1%) of HIV$^+$/AIDS patients was <200 cells/ μ L (P<0.000 1). **Conclusions:** Seroprevalence of latent toxoplasmosis in HIV patients is high, therefore the prevention of toxoplasmic encephalitis, administration of primary prophylaxis with co-trimoxazole to all HIV$^+$/AIDS patients are necessary.

1. Introduction

Toxoplasma gondii(T. gondii), an obligate intracellular protozoan, is one of the most prevalent protozoa in man and livestock[1] and widely distributed around the world[2]. The prevalence of serologic evidence of *T. gondii* infection varies depending on geographic areas and population group. *T. gondii* is horizontally transmitted to humans by accidental ingestion of oocysts in water, food or soil contaminated with cat's feces, or by eating raw or undercooked meat containing cysts[3]. Most infections in immunocompetent humans are asymptomatic and in up to 10% of infected individuals cervical lymphadenopathy or ocular disease occur[4]. *T. gondii* can also cause severe encephalitis via acute infection or reactivation of latent infection among immune-suppressed persons, including those with acquired immunodeficiency syndrome, those with immunosuppressive cancer, and transplant recipients on immunosuppressive drugs. Toxoplasmosis is the most frequent severe neurologic infection among persons with acquired immunodeficiency syndrome[4,5].

Toxoplasmic encephalitis usually occurs in HIV-infected patients with CD4 T-cell counts<100/ μ L[6]. In Iran, up to 50% prevalence of *Toxoplasma* infection has been estimated in different risk groups[7]. Although latent *Toxoplasma* infection is an important problem among HIV patients, it has not been performed any study in north of Iran. Therefore, this study conducted to determine the seroprevalence of anti-*T. gondii* IgG and IgM antibodies in HIV/AIDS patients and HIV-uninfected subjects in this area.

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2. Materials and methods

This cross sectional survey was carried out on 78 healthy and 62 HIV$^+$/AIDS individuals in northern Iran between September 2007 and October 2008. This study was previously assessed and approved by the Ethics Committee at the Mazandaran University of Medical Sciences.

2.1. Collection and examination of blood samples

Five mL of Blood samples were collected from each person in case and control groups. The subjects gave an informed consent. Determination of CD4$^+$ counts was performed by flow cytometry including whole blood samples collected in EDTA from 78 normal and 62 HIV$^+$/AIDS individuals. Immunophenotyping of the lymphocyte subsets was done by using three color flow cytometric methods (partech, Germany). The blood samples were stained by using FITC conjugated mouse anti-human CD4 (clone MT 310) and mouse anti-human CD3 (clone UCHTI) conjugated with RPECYS monoclonal antibodies. All reagents were purchased from Dakocytomation (Dakocytomation, Denmark). The serum separated from blood samples was stored at -20 ℃ until use. The presence of antibodies to *T. gondii* was determined by conventional ELISA technique, according to the manufacturer,s instructions (EUROIMMUN, D-23560 Lubeck. Seekamp 31. Germany). The optical density of IgG antibody titers were read at 490 nm using automatic ELISA reader (SPECTRA, Molecular Devices, USA). Sera with ⩾20 IU/mL and ⩾100 IU/mL were considered positive for anti-*T. gondii* immunoglobulin IgG and IgM antibodies, respectively.

2.2. Statistical analysis

SPSS software 16 was used for statistical analysis. Possible association were identified using the Chi-square and Fisher's exact statistical tests at a significant level of 5%.

3. Results

A total of 140 individuals were enrolled in the study. Sixty two HIV$^+$/AIDS patients (case group) and 78 without clinical signs (control group) were examined for anti-*T. gondii* IgG and IgM antibodies.

The age range of HIV$^+$/AIDS infected participants was 17 to 58 years with a median of (32.6±7.4) years and the mean age of HIV negative individuals was (32.7±9.5) years (range 12-62 years).

Forty eight out of 62 (77.4%) HIV positive/AIDS serum samples were found positive for anti-*T. gondii* IgG antibody, compared with 59 among 78 (75.6%) HIV negative samples from the same area (P>0.05). Except age groups ⩽ 20 (n=1) and ⩾51 years (n=1), prevalence of IgG antibody was higher in age group of 41-50 years old. There was no statistical significant difference among age groups (Table 1).

Six out of 62 (9.7%) HIV$^+$/AIDS patients showed anti-*T. gondii* IgM antibody in their serum samples, compared with 7 among 78 (0.9%) HIV negative samples (P>0.05). Frequency of IgM antibody concerning age has been shown in Table 1.

CD4$^+$ T-cell count was done in all HIV-positive and control people. The mean of CD4$^+$ counts in HIV$^+$/AIDS was (430.8±182.3) cells/μL and in control group was (871.0±243.3) cells/μL (P<0.01).

Ninety two percent of people in control group and 25.5% of HIV$^+$/AIDS patients had a CD4$^+$ T lymphocyte count of ⩾500 cells/μL (P<0.000 1). CD4$^+$ estimation in 5 (11.1%) of HIV$^+$/AIDS patients was <200 cells/μL (P<0.000 1) (Table 2).

Table 1
Frequency of anti-*T. gondii* IgM and IgG antibodies in age groups [n(%)].

Age groups (Years)	IgM$^+$		IgG$^+$	
	Case	Control	Case	Control
⩽20	1 (100.0)	1 (10.0)	1 (100.0)	8 (80.0)
21-30	1 (5.3)	2 (10.6)	13 (68.4)	16 (84.2)
31-40	3 (9.7)	3 (9.7)	24 (77.4)	20 (64.5)
41-50	1 (10.0)	1 (6.3)	9 (90.0)	14 (87.5)
⩾51	0 (0.0)	0 (0.0)	1 (100.0)	1 (50.0)
Total	6 (9.7)	7 (8.9)	48 (77.4)	59 (75.6)

Table 2
Frequency of CD4$^+$ Tcells counts in HIV$^+$/AIDS patients (case) and healthy people (control) by IgG and IgM positive against *T. gondii* [n(%)].

Antibodies	<200		200-499		⩾500		Total	
	HIV$^+$	HIV$^-$	HIV$^+$	HIV$^-$	HIV$^+$	HIV$^-$	HIV$^+$	HIV$^-$
IgG$^+$	5 (11.1)	0 (0.0)	29 (64.4)	4 (8.0)	11 (24.4)	46 (92.0)	45 (100.0)	50 (100.0)
IgM$^+$	0 (0.0)	0 (0.0)	4 (66.7)	0 (0.0)	2 (33.3)	4 (100.0)	6 (100.0)	4 (100.0)

Some samples of case and control groups were not evaluated for CD4 counts.

4. Discussion

In this study, the seroprevalence rates of anti-*T. gondii* IgG antibody due to reactivation of a latent infection among HIV$^+$/AIDS patients and healthy people were 77.4% and 75.6%, respectively. Individuals in both groups had similar exposure to *T. gondii* infection, as reported by other researches[8-10]. The high prevalence of anti-*T. gondii* IgG antibody in these individuals was like the results of previous surveys carried out on the pregnant women of Mazandaran Province, Iran[11]. In Iran there are few reports about prevalence of anti-*T. gondii* antibodies in HIV+/AIDS people. Mardani *et al*[12] in Ghom Province reported the same prevalence (61.33%), but Davarpanah *et al*[13] in Shiraz, southern Iran and Afrasiabian *et al*[14] in Kurdistan Province, north west of Iran reported low prevalence rates (18.2% and 46.9%, respectively). The high seroprevalence of latent Toxoplasma infection among individuals of this area is due to consumption of raw or insufficiently cooked meat, proper climate (high humidity) for survival of the oocyst of *T. gondii* and abundance of cats that contaminate the environment. In contrast, the lower prevalence might be related to climatic condition in Kurdistan (cold weather) and Shiraz (warm and dry weather).

There is wide geographic variation in the prevalence of latent *T. gondii* infection. Studies from different continents such as Latin America, Europe, Asia, and Africa have reported a range of prevalence estimates of 30%-75%, whereas prevalence estimates from USA studies have had a range of 3%-42%[9]. However, this seroprevalence was much higher than some countries *e.g.* 15% in Washington, D.C., USA[9], 44.8%-51.2% in Malaysia[6,15], 5.4% in Japan[16], and 50% in Ethiopia[10]. The differences could be due to the geographical distribution, feeding habits of eating raw or badly cooked meat, diagnostic techniques, or sample size, and foods contaminated with cat feces containing *T. gondii* oocysts.

Since presence of IgG antibodies to *T. gondii* represents chronic infection and is an index for development of toxoplasmosis, therefore serological tests help us to identify those HIV$^+$/AIDS patients who have IgG antibodies to *T. gondii* and are at risk for development of *Toxoplasma* encephalitis (TE). TE represents an important opportunistic infection in HIV$^+$/AIDS patients[16]. Almost all AIDS patients with TE have detectable anti-*T. gondii* IgG antibodies in their serum. TE in a negative *T. gondii* IgG antibody test is less likely. Therefore situation of IgG antibody should be determined in all HIV$^+$/AIDS patients. Seropositive patients need to start oral treatment for toxoplasmosis prophylaxis and without it almost one-third of these patients develop toxoplasmosis[17].

In the USA, it is estimated that 20% to 47% of all the patients with HIV develop encephalitis caused by toxoplasmosis and 25% to 50% in Europe and Africa[17,18]. The estimated disease is related to the incidence of toxoplasmosis in each area, which is higher in France[19] and Brazil[20], to the absence of chemoprophylaxis[21] and to low CD4$^+$ counts[22], specific strain virulence[23] or genetic background[24]. TE is reported to occur at <200 cells+high IgG tites $\geqslant$150 i.u./mL in France[25], <100 cells/μL in USA[26-29] and 35 times more common in those with CD4$^+$<50 cells/μL in Edinburg[30]. In this study the mean CD4$^+$ count in HIV$^+$/AIDS patients was (430.8±182.3) cells/μL blood and 5/45 patients with anti-*T. gondii* IgG antibody showed CD4 count<200/μL; therefore the risk observed in our study was 11.1%. These patients would have a higher chance to develop toxoplasmic encephalitis than others who had CD4 count>200 cell/μL. In clinical practice, CD4 cell count is considered to be a prognostic or risk factor to monitor the progression of HIV infection.

In this survey, 9.7% HIV$^+$/AIDS patients showed anti-*T. gondii* IgM antibody. Afrasiabian *et al*[14] in Kurdistan also reported the same prevalence (10.9%). But Mardani *et al*[12] in Ghom Province showed that no person had anti-*T. gondii* IgM antibody. Ramirez *et al*[31] in their study found only one case with IgM antibody (1.0%). Of course IgM antibody test play no role in the diagnosis of toxoplasmosis in patients with HIV, because the disease is usually due to reactivation of a dormant cyst.

In conclusion, in this survey the seroprevalence of chronic (latent) toxoplasmosis was high and similar among HIV$^+$/AIDS patients and healthy people. Therefore, we propose that all HIV$^+$/AIDS patients have to be tested for *T. gondii* antibody and administration of primary prophylaxis with co-trimoxazole to all HIV$^+$/AIDS patients with a CD4 cell count of less than 200 cells/μL is necessary.

Conflict of interest statement

We declare that we have no conflict of interest.

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