Short Communication

J Babol Univ Med Sci
21; 2019. P:140-146

Molecular and Serological Techniques to Determine the Acute and Chronic Phase of Toxoplasmosis in HIV Patients

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J Babol Univ Med Sci; 21; 2019; PP: 140-6
Received: July 6th 2018, Revised: Dec 4th 2018, Accepted: Dec 24th 2018.

ABSTRACT

BACKGROUND AND OBJECTIVE: Toxoplasma gondii is associated with several complications including neurological problems, ocular damage and encephalitis in immunodeficiency individuals. Early diagnosis of this infection can lead to better management of this disease. Therefore, this study was conducted to determine the presence of Toxoplasma gondii with two serologic and molecular methods in HIV-infected individuals.

METHODS: In this cross-sectional study, 102 male patients with HIV with a mean age of 40 ± 9.2 years were examined. The serum sample was used for ELISA to determine the acute and chronic phase and cellular samples using Real Time-PCR for determining the acute phase of the disease. The relationship between age groups and the HIV transmission pathway, as well as the age group, was compared with the results of the Toxoplasma gondii test.

FINDINGS: Out of 102 samples tested for IgM anti-Toxoplasma gondii antigen by ELFA, all (100%) samples were negative, but for anti-IgG anti-parasite, 44 samples (43.1%) were positive and 58 Sample (56.9%) was negative. Out of 102 samples tested by RT-PCR, all (100%) samples were negative for Toxoplasma DNA. There was a statistically significant relationship between age groups and transmission pathways ($p<0.001$), as well as between age groups with anti-Toxoplasma gondii IgG levels ($p<0.001$).

CONCLUSION: According to the results of this study, the use of IgM-ELFA and PCR-RT methods for the diagnosis of acute phase and IgG-ELFA in the chronic phase of the disease is important. With the diagnosis of chronic form of toxoplasmosis, preventive treatments can be used in HIV+ patients.

KEY WORDS: *HIV, Toxoplasma Gondii, Serological and Molecular Methods*.

Please cite this article as follows:

Shokri F, Abbaszadeh R, Rostamnezhad M, Heidari Digesara S, Marandi N, Bairami Kuzehkanan A, Abolhassani M, Gharavi MJ. Molecular and Serological Techniques to Determine the Acute and Chronic Phase of Toxoplasmosis in HIV Patients. J Babol Univ Med Sci. 2019;21:140-6.

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Introduction

Toxoplasma gondii is an intracellular compulsory parasite of the Apicomplexa family that is scattered around the world. In addition to humans, this parasite infects a large number of mammals and replicates in a wide range of host cells (1). In humans, congenital toxoplasmosis causes dangerous complications, such as abortion and death, and also causes neurological problems, eye damage and encephalitis in people with immune deficiency (AIDS) (2-4). The final host of this parasite is cat, which is the sexual cycle in the body of these animals and the intermediate hosts is human and other mammals (5). Toxoplasma infection includes acute and chronic infections, with more symptoms occurring in the acute phase (6). Patients with immune deficiency, including those with HIV, are the most common clinical manifestations of central nervous system, heart, lung and eye involvement. One of the causes of death in people with AIDS is the activation of chronic toxoplasmosis infections in the central nervous system, which is one of the most common causes of encephalitis in these patients (7).

Despite the importance of toxoplasmosis, few studies have been conducted on the prevalence of toxoplasmosis in HIV patients, and all studies have also examined the prevalence of toxoplasmosis in serum (8-10). The diagnosis of toxoplasmosis, especially in immunodeficiency patients, is often accompanied by false negative results (11,12), which is often associated with late-onset diagnosis and treatment (13). Based on the results of the studies, the use of molecular methods such as PCR has a higher value for the detection of Toxoplasma gondii from blood, amniotic fluid and pairs (14,15). In a study using RT-PCR technique for the detection of toxoplasmosis, it was found that this method has high sensitivity and specificity for diagnosis (16). Because toxoplasma harm irreparably the patients with HIV, which in some cases can lead to death, early diagnosis of this infection can lead to better management of the disease, so this study aims to Detection of Toxoplasma gondii by ELFA method was performed for the presence of IgM and IgG antibodies and also the molecular method of Real-time PCR for the presence of DNA of Toxoplasma gondii. Then, the comparison of these two methods for diagnosis was done in people with HIV.

Methods

Study population: This cross-sectional study was approved by the Committee on Ethics of the Alborz University of Medical Sciences with the code of IR. ABAUMS. PSRC. REC 1396. 4038 cases were performed on HIV patients in Ghezel Hesar prison of Karaj. By observing ethical considerations and ensuring confidentiality of information and obtaining written consent from patients, HIV-infected people were enrolled in a census.

All subjects were male and their age range was between 20 and 60 years old. The criteria for entering this study were the informed consent of HIV+ patients and exclusion criteria including lysed blood samples, death, transference to prison or other cities and unwillingness to cooperate in the study. Using GPower software Version 3.1.9.2 and according to Rezanezhad et al. (17), with a prevalence of 21.1%, a 95% confidence level and a 5% error, the sample size was determined to be 128. But in this study, considering that all HIV+ prisoners were enrolled in the study, only 102 of them were willing to participate in the study, which were examined.

From each patient 4ml blood was collected and entered into an EDTA tube, then the serum sample and their blood cells were separated and serum samples were collected for serological examination using ELFA method. Serum samples were transferred to the laboratory serology for ELFA test to check the presence of anti-Toxoplasma gondii antibodies. Also the blood cell sample was transferred to molecular labratory for DNA extraction by QIAGEN QIAamp DNA Mini Kit, and DNA was extracted with high purity and then the Real Time-PCR was used to confirm the presence of Toxoplasma gondii DNA.

SEROLOGICAL METHODOLOGY: The Mini VIDAS and the Biomerieux specialty kit were used to conduct ELFA tests. The kit contains strip (STR), with various parts of which include:

Well 1: sample,
Well 2: Serum diluents,
Well 3: Pre-Wash Buffer
Wells 4-5-7-8: Washing Buffer
Well 6: Conjugate,
Well9: empty hole,
Well 10: Cuvette with Substrate.
At first, the STR was brought to room temperature and then 100 µl of the sample was added to well No. 1. Each STR was for a sample and STR was placed in the device's location.

Molecular analysis: DNA was extracted from blood for molecular detection of Toxoplasma gondii. A primer was designed for toxoplasma gondii B1 gene for 529bp repeat sequences.

The primer sequence bound to the 529bp element contains 5'-CACAGAAGGG ACAGAAGT-3 'and 5'-TCGCCTTCATCTACAGTC-3'. The length of the duplicated part between these two primers is bp94. The temperature and time cycles during RT-PCR included repeating 30 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds, and finally final extension for 5 minutes at 72 °C. Real Time device of Sacace Company was used and positive and negative control was used to determine the accuracy of the reactions.

Epidemiological parameters: In this study, epidemiological parameters such as transmission pathways, age and clinical diagnosis of toxoplasmosis were investigated. Data were analyzed by SPSS software version 18 using descriptive statistics (including mean, standard deviation and percentage) and analytical (Chi-square test). P<0.05 was considered significant.

Results

Serologic method: From 102 samples tested for IgM Anti-Toxoplasma gondii by ELFA, all (100%) samples were negative, but for IgG Anti-Toxoplasma gondi 44 (43.1%) samples were positive and 58 (56.9%) samples were negative (Table 1).

Molecular Method: Out of 102 samples examined by RT-PCR, all (100%) samples were negative for Toxoplasma DNA. In Fig. 1, the results obtained from serological and molecular methods were compared. According to our study, the highest rate of transmission of HIV infection was injection with infected syringe (54.9%), and transmission through the infected knife (98%) had the lowest transmission rate (Table 2).

Table1. Comparison of frequency of Toxoplasma gondii infection with both serological and molecular methods

Results / Method	Positive N(%)	Negative N(%)	Total N(%)
RT-PCR	0(0)	102(100)	102(100)
ELFA-IgM	0(0)	102(100)	102(100)
ELFA-IgG	44(43.1)	58(56.9)	102(100)

The mean of subjects age was 40±9.2 years. Chi-square test showed significant correlation between age group and HIV transmission way (p<0.001), as well as age group with IgG Anti-Toxoplasma gondii (p<0.001). The highest rate of HIV infection was observed in the age group of 30-39 years (41.2%) (Table 3). Out of the studied subjects, in addition to HIV infection 27 samples were HCV positive (26.4%), 4 were TB positive (3.9%), 1 was hepatitis B positive (HBV) (0.98%), and 3 people also had hepatitis C (HCV) and TB (94.2%) at the same time.

Table2. Frequency of AIDS transmission ways in studied subjects

Transmission way	N(%)
Injection	56(54.9)
High risk relationship	34(33.33)
Tattoos	9(8.83)
Infected Gillette	2(1.96)
Infected knife	1(0.98)
Total	102(100)

Table3. Outbreak of AIDS in the studied age groups

Age group	HIV+ people N(%)
20-29	33(32.4)
30-39	42(41.2)
40-49	19(18.6)
>50	8(7.8)

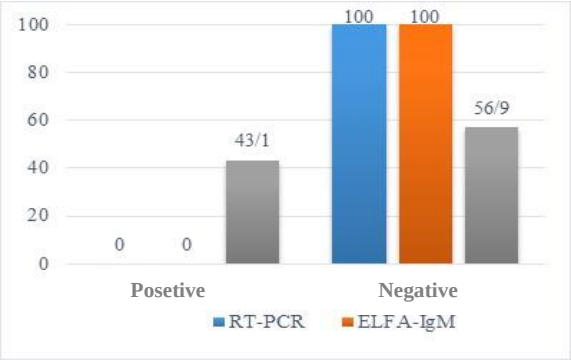

Figure 1. Distribution of toxoplasma gondii infection distribution by serological and RT-PCR methods

Discussion

Based on the results of this study, the ELFA method, anti-Toxoplasma IgM was 0% and IgG antibody was 43.1%, which indicates previous infection with this parasite. This study also indicates that the ELFA-IgM and PCR-RT methods for diagnosis of acute phase can be preferable. Shafiei et al., in Mashhad, with ELISA and Western blotting, reported an IgG and IgM seroprevalence in HIV infected individuals of 38.1% and 2.5% respectively (10). Rahimi et al., in Mazandaran, reported the prevalence of IgG Anti-Toxoplasma serotype in HIV+ infected individuals' about96.3%. Meanwhile, none of the samples was positive for IgM (18).

Ahmadpour et al. reported in a meta-analysis study that seroprevalence of toxoplasma in HIV+ cases in Iran were 50.5% (19). Davarpanah et al. in Shiraz reported the prevalence of anti-Toxoplasma IgG in HIV+ patients with ELISA method (18.26%) (9). In a study conducted by Chemoh et al. in Thailand, the prevalence of anti-Toxoplasma IgG in the HIV+ population was 36.3% (20). Walle et al., in North-Ethiopia, reported the prevalence of anti-Toxoplasma iIgG and IgM n HIV+ patients by ELISA about 87.4% and 10.4% respectively (21,22). By reviewing a number of previous studies of Toxoplasma gondii in HIV+, we conclude that the prevalence of toxoplasmosis is different in different regions of the world and even in Iran, and this difference is due to the level of health, the consumption of raw meat, the high humidity, the contamination of the environment by cat as the final host of this parasite and so on... (18). As in the present study, there is a significant relationship between age and anti-Toxoplasma antibody levels. In Jones et al., It was also found that there is a relationship between anti-toxoplasm antibodies and the age of the person (23). One of the features of this study is that it was first mentioned in Alborz Province. Alternatively, the selection of the ELFA serology method is more sensitive than ELISA, luminescence and immunofluorescence (24). ELFA's automatic method is evaluated as an appropriate method for its high repeatability, saving time and lower cost, (25). Also RT-PCR technique was used in this study. In this method, a 529pb repeating sequence is used, which has high sensitivity to the detection of toxoplasma DNA (26). Therefore, in this study we can compare two molecular and serological methods for diagnosis of Toxoplasma gondii. According to the results of this study, the prevalence of HIV+ infection by Toxoplasma gondii by IgG-ELFA method was more than PCR-RT, indicating that none of the subjects had acute infection and had contact with the infection before.

Also, the results of IgM-ELFA emphasize that none of the samples has active infection. The presence of anti-Toxoplasma gondii IgG represents a chronic form of the disease and is an indicator of the prevalence of toxoplasmosis. Serologic tests help identify HIV+ patients whose positive IgG antibodies are susceptible to encephalitis (27).

In this regard, a diagnostic method that has high sensitivity and repeatability and results in a short time can be very useful. Negative IgG with negative IgM indicates that the person is not exposed to Toxoplasma gondii and un-immunization. It should be noted that acute infection in HIV+ people who did not have previous exposure to Toxoplasma gondii was not well controlled and these would be susceptible to secondary complications (28).

Therefore, the serological test, especially ELFA, which is very suitable for IgM assays, is also important in identifying people who have not been exposed to this parasite. According to the results of this study and similar studies, toxoplasma gondii is one of the most common parasites among HIV+ patients and has lethal side effects. Therefore, the use of IgM-ELFA and PCR-RT methods for diagnosis of acute phase as well as IgG-ELFA serologic for diagnosis of chronic formof the

disease is important to screen patients with HIV+. With the diagnosis of chronic toxoplasmosis, preventive treatments can be used to prevent complications such as encephalitis in HIV+ patients.

Acknowledgment

Hereby, we would like to thank the authorities of Ghezel Hesar jail as well as laboratory personnel to cooperation in the investigation.

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