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# *Toxoplasma gondii*: The Prevalence and Risk Factors in HIV-Infected Patients in Fars Province, Southern Iran

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## Abstract

**Background:** *Toxoplasma gondii* is a zoonotic protozoan that threatens the health of the people infected with human immunodeficiency virus (HIV). HIV-positive subjects are at risk of cerebral toxoplasmosis.
**Objectives:** The current study was designed to find out the seroprevalence of toxoplasmosis in HIV-infected individuals in Fars province, in the South of Iran.
**Methods:** This cross-sectional study was performed on 251 HIV-infected individuals referred to Shiraz HIV/AIDS Research Center in Fars province, Southern Iran, in 2016. Blood samples (5 mL) were collected from each individual and sera as well as buffy coat were isolated from each sample. Demographic and HIV-associated data were obtained from the patients' medical records. Anti-*Toxoplasma* antibodies (both IgG and IgM) were determined by ELISA, using a commercial kit. In addition, PCR, targeting a 529 bp gene of *T. gondii* was performed on the patients' buffy coats for detection of *Toxoplasma* DNA.
**Results:** Anti-*Toxoplasma* antibodies were detected in the sera of 42 out of 251 (16.7%) HIV infected patients. Of these, 39 cases (15.5%) were seropositive only for IgG and 3 (1.2%) were positive only for IgM. Seropositive subjects mainly belonged to 40 - 49 year age group. None of the subjects were positive for *Toxoplasma* DNA when evaluated by the PCR. No significant associations were found between *Toxoplasma* seropositivity and gender, age, and CD4+ cell level ($P > 0.05$).
**Conclusions:** Findings of this study demonstrated a significant rate of seroprevalence of *Toxoplasma* in HIV infected subjects in Fars province, Southern Iran. The seropositive cases are at risk of *Toxoplasma* reactivation and subsequent cerebral encephalitis. Treatment and also prevention of toxoplasmosis in HIV positive people should be considered.

*Keywords:* HIV, Polymerase Chain Reaction, Risk Factors, Seroepidemiologic Studies, Toxoplasmosis

## 1. Background

Opportunistic infections are one of the most critical challenges in the management of HIV infected individuals which can cause morbidity and mortality in these patients. *T. gondii*, as a zoonotic protozoan with worldwide distribution, is one of the life-threatening obligate opportunistic pathogens in immunosuppressed patients (1, 2). Reactivation of latent toxoplasmosis appears in the form of encephalitis and also disseminated toxoplasmosis.

*T. gondii* is a food-borne protozoan, which usually infects humans through consumption of contaminated water and food, as well as through placental transmission (3, 4). Prevalence of *T. gondii* infection in HIV-positive subjects in different areas of the world depends on people's dietary and cultural food habits as well as the climatic condition of the area. The global seropositivity of *T. gondii* among the HIV population is reported to be 35.8% (5). A higher rate of seropositivity for *T. gondii* in HIV-infected individuals have been reported from Brazil (80.0%), Morocco (62.1%), and Iran (55.1%) (1, 6, 7). Both HIV and *Toxoplasma* infections are challenging health problems in Iran (4, 8-13). Coinfection of HIV/*Toxoplasma* in Iran ranged from 20% to as much as 95% in some areas (14-16). The highest rate of seropositivity to *Toxoplasma* in HIV-infected subjects (96.3%) has been reported from the Mazandaran province, in the north of the country, while lower prevalence has been reported from Tehran (49.75%) and also from Kurdistan province (19.1%), which is in the west of Iran (14-17).

The incidence of cerebral toxoplasmosis is an important health concern in HIV-positive patients in areas where

co-infection of HIV/*Toxoplasma* is high. In a retrospective study on 208 HIV/AIDS patients in the south of Iran, four (10.4%) of the patients were reported to have *Toxoplasma* encephalitis (18). High prevalence of *Toxoplasma* infection in sheep and goat meats from the Fars province, southern Iran (19), as well as the abundant population of cats in the region increase the risk of toxoplasmosis in general population as well as in the HIV-infected individuals. The current study was justified by the lack of information regarding the prevalence of *Toxoplasma* among the HIV-infected patients in Fars province, in the south of Iran. Findings of this study might be used for the improvement of *Toxoplasma* surveillance in HIV-infected individuals in Iran and also in other countries within the region.

## 2. Objectives

The current study aimed to determine the seroprevalence of *Toxoplasma* and also to detect *Toxoplasma* DNA in buffy coats of HIV infected patients in the Fars province in the southwest of Iran.

## 3. Methods

### 3.1. Study Population

This cross-sectional study was performed on 251 HIV-infected individuals referred to Shiraz HIV/AIDS Research Center in the Fars province, southern Iran, from May until September 2016. This governmental referral center is affiliated to the Shiraz University of Medical Sciences and provides diagnostic and treatment services for HIV patients, which referred to the center from the entire region. The criteria for selecting the patients were having a positive ELISA and also western blotting tests for HIV and having a complete medical record. Those subjects with incomplete records were excluded from the study. Ethical Committee of Shiraz University of Medical Sciences approved the study (Ethical code: IR.SUMS.REC.1394.S858). The study entirely considered the key Ethical issues such as obtaining informed consent, building trust, and imposing no maltreatment on the participants. Fars province is considered as the focus of several important parasitic diseases in Iran (20-25).

### 3.2. Sampling

The sample size was calculated considering the seroprevalence of reference of 18%, confidence level of 95%, and a marginal error of 0.05, using the sample size calculation formula for cross-sectional studies. Considering these criteria, the required sample size was found to be 226 where we collectively enrolled 251 HIV infected patients that met the study criteria.

Five mL of blood samples were taken from each patient in K2-EDTA tubes (Pishtaz Teb Zaman Diagnostics, Iran). The fresh whole blood was centrifuged for 10 minutes at 800 g, and the sera and buffy coat layer were removed. While maintaining the cold chain, the samples were transferred to the Immunoparasitology Laboratory at Shiraz University of Medical Sciences (SUMS) and kept at -20°C until use. Demographic information along with HIV-associated data was obtained from the patient's medical records.

### 3.3. Detection of Anti-Toxoplasma Antibodies

The presence of anti-*Toxoplasma* IgG and IgM antibodies were assessed by a commercial ELISA kit (Acon Biotech, Hangzhou, China), based on the manufacturer's instructions. The level of IgG and IgM antibodies were measured at 450 and 630 nm, using a plate reader (BioTek; Winooski, Vermont, USA). Index value > 1.1 was regarded as positive. Index values less than 0.9 regarded as negative while those values between 0.9 and 1.1 were regarded as equivocal. Samples within the equivocal zone were re-tested.

### 3.4. PCR for Detection of Toxoplasma DNA

DNA was extracted from the buffy coat of the blood samples as previously described (10). Briefly, 250 $\mu$L of TNNT buffer (0.5% Tween 20, 0.5% Nonidet P-40, 10 mM NaOH, and 10 mM Tris pH: 7.2) and 10 $\mu$L of proteinase K (10 $\mu$g/mL) was added to the buffy coat and incubated overnight at 56°C. Then the phenol-chloroform extraction method was used for subsequent DNA extraction. The forward TOX4-(5'- CGCTGCAGGGAGGAAGACGAAAGTTG) and reverse TOX5-(5'- GCTGCAGACACAGTGCATCTGGATT) primers (Bioneer, Daejeon, Korea) were used for the amplification of a 529 base pair fragment of *Toxoplasma*, as previously described (17). Briefly, 10 ng of template DNA and 10 pmol of each primer were added to a 1x Taq DNA polymerase master mix (Ampliqon, Odense, Denmark) in a final volume of 25 $\mu$L. The PCR was performed with the Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany) with the cycle program of 94°C for 5 minutes, 35 cycles at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and an ending extension at 72°C for 10 minutes. The PCR products were analyzed by electrophoresis on a 1.5% agarose gel and stained with GelRed nucleic acid gel stain (GelRed®, Sigma-Aldrich, USA).

### 3.5. Statistical Analysis

Collected data were analyzed by SPSS statistical software, version 18.0 (SPSS Inc, Chicago, Ill., USA). Descriptive statistics were performed to indicate the frequencies of the data. The association between seroprevalence of toxoplasmosis and qualitative variables were assessed by Chi-square test. The significance level was defined as 0.05.

## 4. Results

Subjects of the study were 251 HIV positive patients including 158 (63.5%) males and 91 (36.5%) females (two missing gender value), aged 14 to 83 years old. The mean age of the subjects was 40.8 ($\pm$ 9.72), and most of the cases were in the age groups of 30 - 49 years. The mean CD4+ cell level of the patients was 403 cell/$\mu$L; with 7.6% less than 200 cell/$\mu$L.

### 4.1. Seroprevalence of Toxoplasmosis Among the HIV-Positive Subjects

Anti-*Toxoplasma* antibodies were detected in the sera of 42 out of 251 (16.7%) HIV infected patients. Out of these 42 subjects, 39 cases (15.5%) were seropositive for IgG and 3 (1.2%) were positive for IgM, whereas none of the patients were simultaneously positive for IgG and IgM antibodies. The seroprevalence rate was insignificantly higher in males (18.4%) in comparison with the females (13.2%). The highest rate of seropositivity, based on the age groups and the CD4+ level, was observed in the age group of 50 - 59 years and patients with a CD4+ level between 400 to 600 cells/$\mu$L. None of the samples was positive for *Toxoplasma* DNA when evaluated by PCR. No significant associations were found between *Toxoplasma* seropositivity and gender, age, and CD4+ cell level. Table 1 shows the demographic characteristics and relative seropositivity to *Toxoplasma* in the HIV-positive subjects in this study.

## 5. Discussion

The current study investigated the prevalence of *Toxoplasma* among HIV-positive patients in the south of Iran, using serological and molecular (PCR) methods. The overall seroprevalence of toxoplasmosis in HIV positive cases in the study was 16.8%. Most of the seropositive subjects (15.8%) were positive for IgG, which indicates a previous infection. These patients were at risk of *Toxoplasma* encephalitis when their CD4+ level dropped below the 100 cells/$\mu$L, which is a threshold for reactivation of latent toxoplasmosis in HIV positive patients. Considering this point, seropositivity to *Toxoplasma* might have a predictive value for the primary diagnosis of *Toxoplasma* encephalitis. Moreover, the presence of IgM antibodies against *T. gondii* in 3 patients may indicate a recent infection, although in some patients, IgM antibodies may persist at a detectable level for a relatively long time, after *Toxoplasma* infection.

In this study, *Toxoplasma* DNA was not detected in the buffy coats of either seropositive or seronegative cases. The absence of *T. gondii* DNA in the buffy coat of HIV-infected individuals may be linked to the very low level of concentration of *T. gondii* DNA in these patients. This finding is somewhat similar to the two previous reports among HIV/AIDS patients from Sanandaj, west of Iran, and Jahrom, south of Iran, where only one patient tested positive for *T. gondii* DNA in each of these studies (14, 17). Protocol of DNA extraction, the PCR target gene, the employed primers, and even the volumes of blood samples can influence the sensitivity of the PCR assay for the detection of *Toxoplasma* DNA in a given sample, including buffy coat (26).

Studies on *T. gondii* HIV co-infection in different regions of Iran demonstrated a rate of 18% up to 96% (14, 16, 17). The seroprevalence rate of 16.8% in the present study is close to a previously conducted retrospective study in the south of Iran, based on patients' records, which reported a seroprevalence of 18.2% for toxoplasmosis (18). The seroprevalence rate in the current study is also close to the study of Rostami et al. (17), in Sanandaj, Kurdistan province, and west of Iran, which reported a 19.1% seroprevalence rate for *Toxoplasma* in HIV positive patients. Dry climate in Sanandaj and semi-dry in Fars province could be attributed to the close prevalence rate of *T. gondii* infection in these two areas of Iran.

In comparison with other studies, the prevalence rate of *Toxoplasma* in the current study was lower than some of the studies on the HIV-positive population in Iran. For instance, two serological studies on HIV-positive infection in the northern part of Iran reported a prevalence rate of 96.3% and 77.4% (11, 16). In other studies from Tehran and Mashhad, prevalence rates of 49.75% and 47.7% have respectively been reported (15, 27). The prevalence rate of *Toxoplasma* in our study was also lower than some of the studies in other countries including Ethiopia (93.3%) (28), Ghana (57.6%) (29), in southern Thailand (36.3%) (30), and Indonesia (77.2%) (31).

Different factors may affect the variations in seroprevalence rate of toxoplasmosis in different areas of the country which are; food and cultural habits in a community, people lifestyles, climatic condition, geographical location, the density of cats in the environment, and also the

**Table 1.** Demographic Features of HIV Infected Patients and Relative Seropositivity to *Toxoplasma* in Fars Province, Southern Iran

| Characteristics | No. (%) | Seronegative (IgM or IgG) | Seropositive (IgM or IgG) | Seropositive (IgG) | Seropositive (IgM) |
|---|---|---|---|---|---|
| **Gender** | | | | | |
| Male | 158 (63.5) | 129 (81.6) | 29 (18.4) | 26 (16.5) | 3 (1.9) |
| Female | 91 (36.5) | 79 (86.8) | 12 (13.2) | 12 (13.2) | 0 (0.0) |
| **Age (y)** | | | | | |
| < 30 | 29 (11.6) | 26 (89.7) | 3 (10.3) | 3 (10.3) | 0 (0.0) |
| 30 - 39 | 97 (38.6) | 85 (87.6) | 12 (12.4) | 11 (11.3) | 1 (1.0) |
| 40 - 49 | 93 (37.1) | 74 (79.6) | 19 (20.4) | 17 (18.3) | 2 (2.2) |
| 50 - 59 | 15 (6.0) | 11 (76.5) | 4 (26.6) | 4 (26.7) | 0 (0.0) |
| > 60 | 17 (6.8) | 13 (6.2) | 4 (23.5) | 4 (23.5) | 0 (0.0) |
| **CD4+ level[a]** | | | | | |
| $\leq$ 200 | 19 (7.6) | 15 (78.9) | 4 (21.1) | 4 (21.1) | 0 (0) |
| 201 - 400 | 45 (17.9) | 37 (82.2) | 8 (17.8) | 7 (15.1) | 1 (2.2) |
| 401 - 600 | 114 (45.4) | 96 (84.2) | 18 (15.8) | 17 (14.9) | 1 (0.9) |
| $\geq$ 601 | 73 (29.1) | 61 (83.6) | 12 (16.4) | 11 (15.1) | 1 (1.4) |

Abbreviations: ELISA; enzyme-linked immunosorbent assay; IgM: Immunoglobulin M; PCR: polymerase chain reaction.
[a] CD4+: CD4+ T cells.

sensitivity of the employed serological assays.

Prevalence of *Toxoplasma* infection in this study could be attributed to contamination of meat and also food habits in the area. A high prevalence of *T. gondii* infection was reported in food animals (19), reared turkeys (32), and also in different groups of human population in Fars province where the current study was conducted (13, 33).

The decrease of the CD4+ cell below 200 cells/$\mu$L is considered as one of the most risk factors for reactivation of toxoplasmosis in HIV positive patients, although reactivation of chronic toxoplasmosis in patients with the CD4+ level higher than 200 cells/$\mu$L has also been reported (34). In our study, 7.6% of the subjects had a CD4+ level below 200 cells/$\mu$L. Although no *Toxoplasma* encephalitis was recorded in the patients, they are at risk of *Toxoplasma* reactivation, especially when the CD4+ level dropped below the 100 cells/$\mu$L.

AS far as our knowledge, this is the first study conducted on HIV-infected individuals in Fars province in the south of Iran, using both molecular and serological methods. This can be considered as the main strength of the study. However, the study has several limitations, including the absence of proper clinical features of the patients and not using the real-time PCR, which is a more sensitive molecular method in comparison with conventional PCR for detection of *Toxoplasma* DNA.

### 5.1. Conclusions

Taken together, the findings of this study demonstrated a significant rate of seroprevalence of *Toxoplasma* in HIV infected subjects in Fars province, southern Iran. The seropositive cases are at risk of *Toxoplasma* reactivation and subsequent cerebral encephalitis. Treatment and also prevention of toxoplasmosis in HIV positive people should be considered. Furthermore, *T. gondii* antibodies should be screened in new patients who have recently been diagnosed as HIV patients. Necessary education should be given to HIV infected patients who are seronegative for toxoplasmosis for reducing the risk of acquiring toxoplasmosis.

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## Footnotes

**Authors' Contribution:** Designed the study: Bahador Sarkari and Nasir Arefkha. Collected the samples: Nasir Arefkha, Mona Dehghani, and Masood Afrashteh. Performed the experiments: Nasir Arefkha, Zahra Rezaei,

Mona Dehghani, and Masood Afrashteh. Analyzed the data: Bahador Sarkari and Nasir Arefkha. Wrote the paper: Bahador Sarkari and Nasir Arefkha. All of the authors read and approved the final version of the manuscript.

**Conflict of Interests:** The authors declare that they have no competing interests.

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