Epidemiologic evaluation of toxoplasmosis and leading risk factors in HIV/AIDS patients in Arak City, Iran

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BRIEF REPORT


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ABSTRACT

Background
Toxoplasmosis is a common opportunistic infection that can be fatal in immunocompromised individuals, such as those with HIV/AIDS.

Aims
Considering the rising incidence of HIV/AIDS in human populations worldwide and the high risk of toxoplasmosis among these patients, the current epidemiologic study was conducted to identify the characteristics and leading risk factors of toxoplasmosis among HIV/AIDS patients in Arak City, Marzaki Province, Iran.

Methods
This cross-sectional study was conducted in HIV patients under the care and counselling of the local health centre of Arak City. We included a total of 49 patients with HIV/AIDS who completed a written informed consent form and a two-part questionnaire. Demographic data and information about various risk factors were collected in the questionnaire. Blood samples were collected from each patient. Anti-Toxoplasma gondii IgG and IgM antibody assays and PCR were conducted on serum samples. Logistic regression and chi-squared ($\chi^2$) tests were used for statistical analysis. P values less than 0.05 ($p<0.05$) were considered significant.

Results
Of the study participants, 22.4 per cent were Toxoplasma seropositive, with 20.4 per cent and 2 per cent being IgG- and IgM-positive, respectively. Among the participants, those who had occupational exposure to soil had the highest risk for toxoplasmosis ($p<0.043, OR=7.243$).

Conclusion
The seroprevalence of toxoplasmosis in HIV/AIDS patients is lower in Arak than in the general population in most parts of Iran. This is possibly owing to racial and geographic differences.

Key Words
Epidemiology, Toxoplasma gondii, HIV/AIDS, risk factors

Implications for Practice:

1. What is known about this subject?
There is 25.1 per cent co-infection of toxoplasmosis and HIV in Asia and the pacific. Toxoplasmosis among these people could be fatal.
2. What new information is offered in this report?
In Arak city, in contrast of most parts of Iran the prevalence of toxoplasmosis in HIV+ patients are lower than general population.

3. What are the implications for research, policy, or practice?
All HIV+ patients should be screened for toxoplasmosis infection.

Background
Toxoplasmosis is a zoonotic disease caused by intracellular parasite, Toxoplasma gondii (T. gondii). Human can be infected in two ways, acquire or congenital infection. The course of disease in the body comprises acute and chronic phases. The host immune system converts acute disease to chronic infection and tiny cysts are formed in the host’s tissues. Signs and symptoms of the disease can be observed in the acute phase. Immune deficiency in people with chronic toxoplasmosis can lead to reactivation of the parasite and emergence of dangerous or even fatal conditions, such as encephalitis. A low number of CD4+ lymphocytes in HIV/AIDS patients can lead to increased risk of opportunistic infections or reactivation of chronic infections. Owing to the high prevalence of toxoplasmosis in developing countries, opportunistic forms of toxoplasmosis are higher among immunocompromised people in these countries.

The prevalence of toxoplasmosis in people with HIV/AIDS has been studied in different parts of Iran. In northeastern Iran, the seroprevalence of anti-T. gondii IgG and IgM antibodies has been reported as 38 per cent and 2.5 per cent, respectively. In northern Iran, seroprevalence of anti-T. gondii IgG and IgM antibodies was 96.3 per cent and 0 per cent, respectively, and in southwestern Iran (Ahvaz), the seroprevalence of anti-T. gondii IgG antibody was 73.8 per cent. The result of a systematic review showed that the prevalence of toxoplasmosis was 50.05 per cent among HIV/AIDS patients in Iran. The leading risk factors for reactivation of disease are unknown but immunosuppressive therapy or immunosuppressive diseases may increase the risk of toxoplasmosis reactivation. The prevalence of chronic toxoplasmosis in Arak City has been reported as 24.3 per cent, 38 per cent, and 35.57 per cent, in women, pregnant women and men, respectively. According to these reports, about one-third of the general population is infected with toxoplasmosis in this city.

There is little or no basic information available about toxoplasmosis in HIV positive individuals, and the US centres for Disease Control has targeted toxoplasmosis as a neglected parasitic disease for priority public health action. Therefore, population data on the prevalence rate of toxoplasmosis among those at risk, such as HIV/AIDS patients, will help improve prophylaxis and health planning for those individuals. The aim of this study was to obtain the prevalence of toxoplasmosis among the population with HIV/AIDS in Arak, Iran.

Methods
This cross-sectional study was performed on 49 out of 86 HIV positive patients under the care and counselling of the public local health centre in Arak city, centre of Iran. It should be noted that only 49 patients were willing to participate in this study. All participants provided their written informed consent and completed a two-part questionnaire. Demographic and major risk factor information was collected in the questionnaire. The leading risk factors recorded included a history of injection drug use, blood transfusion, occupational contact with soil, contact with a cat, eating raw or undercooked meat, eating unpasteurized dairy products, and CD4+ cell count.

Antibody assay
Three-millilitre blood samples were collected from each patient. For each sample, the serum was separated by centrifugation at 3000rpm and assessed for the presence of anti- T. gondii IgG and IgM antibodies. Antibody detection was performed using an ELISA kit (Pishtaz Teb Co. Ltd., Iran) according to the manufacturer’s instructions.

Molecular assay
Patient blood cells were examined using molecular methods. Genomic DNA was extracted using phenol-chloroform extraction protocol. Standard primers were used for polymerase chain reaction (PCR), as follows: forward, 5’- CGCTGCAGGGAGGAAGACGAAAGTTG-3’ and reverse, 5’- CGCTGCAGACACGTGCATCTGGATT-3’. Optimization of the PCR reaction was carried out with a Master-Mix kit (CinnaGen Co.), and amplification was performed in a final volume of 25µl on an Eppendorf thermocycler with 5 min incubation at 94˚C, followed by 35 cycles of 30 min at 94˚C, 30 min at 58˚C, 30 min at 72˚C, and a final 10-min incubation at 72˚C. The amplified product was analysed using electrophoresis on a 1 per cent agarose gel. It should be noted that we used T. gondii DNA as a positive control and distilled water as a negative control.
Statistical analysis

Statistical analysis was performed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA). The association between leading risk factors of toxoplasmosis in seropositive and seronegative patients was measured with logistic regression and chi-squared ($\chi^2$) analysis. P values less than 0.05 ($p>0.05$) were considered significant.

Results

Demographic characteristics of the 49 participants in our study were as follows: the mean age was 36 years; most participants were male (65.3 per cent), married (65.3 per cent), and residing in the city (95.9 per cent). Of the participants, 42.9 per cent were unemployed and 32.7 per cent were academically educated. Serological survey of participants showed that 22.4 per cent (11 patients) were Toxoplasma seropositive. Of these, 20.4 per cent (10 patients) and 2 per cent (1 patient) were positive for anti-\( T. gondii \) IgG and IgM antibodies, respectively. A molecular survey of blood samples was negative.

Logistic regression analysis revealed that there was a significant difference between job-related contact with soil and toxoplasmosis in patients ($p<0.043$, OR=7.243). Because of the minor difference, it is considered as a borderline difference, and it may be determined by increasing the sample size. The analysis also revealed that there were no significant differences between the other leading risk factors and toxoplasmosis in these patients ($p>0.05$) (Table 1).

The mean CD4+ cell count was 439.9 cells/µl. Among 26.5 per cent, 38.8 per cent and 34.7 per cent of patients, the CD4+ cell count were <200, 200–499 and >500 cells/µl, respectively.

Among 26.5 per cent of patients, the CD4+ cell count was <200 cells/µl. $\chi^2$ analysis revealed that there was no significant difference between CD4+ cell count and toxoplasmosis among study participants ($p>0.001$).

Discussion

Toxoplasmosis in people who are immunocompetent is benign and self-limiting, but the disease can be disseminated and devastating in immunocompromised individuals.\(^5,12\) Diseases that lead to severe immune deficiency, such as uncontrolled AIDS, can reactivate chronic toxoplasmosis, thereby creating a dangerous health condition.\(^7\) Of the HIV/AIDS patients in this study, 22.4 per cent were Toxoplasma seropositive, with 20.4 per cent and 2 per cent being IgG- and IgM-positive, respectively. Participants who had occupational exposure to soil were at a greater risk for toxoplasmosis ($p<0.05$) than other participants were.

The most common methods used to diagnose toxoplasmosis are limited among immunocompromised individuals because tissue tests are not sensitive, serological tests are unreliable, and PCR cannot always differentiate acute and chronic infection.\(^12\)

A comparison of serological results between HIV/AIDS patients and the general population in different parts of Iran showed that in the most of the regions investigated, the seroprevalence of toxoplasmosis in HIV/AIDS patients is higher than that of general population, except in Shiraz and Arak.\(^19,21\) The reason may be owing to racial and geographic differences because the researchers believe that the prevalence of toxoplasmosis coinfection with HIV is related to these two factors.\(^11,22\)

Conclusion

In the current study, 22.4 per cent of the HIV positive patients were Toxoplasma-seropositive. Given the risk of reactivation of latent toxoplasmosis on the patients, it seems the screening of toxoplasmosis in centers of HIV counselling local health is necessary. Because, in HIV positive patients, serologic tests do not have sufficient ability to diagnose toxoplasmosis, testing different diagnostic methods will be useful in order to find the appropriate method.

References


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PEER REVIEW
Not commissioned. Externally peer reviewed.

CONFLICTS OF INTEREST
The authors declare that they have no competing interests.

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ETHICS COMMITTEE APPROVAL
This thesis was approved by the Ethics Committee of Arak University of Medical Sciences (IR.ARAKMU.REC.1394.315).
Table 1: Analysis of some leading risk factors of toxoplasmosis between negative and positive samples

| Variable                        | HIV/AIDS patients |         |     |     | |             |     |
|---------------------------------|-------------------|---------|-----|-----|--------------------------|-----|
|                                 | Sero + (per cent) | Sero – (per cent) | OR  | CI 95 per cent for OR | P-value |
|                                 |                   |             |     | Lower | Upper |
| Intravenous Drug Use (IDU)      |                   |             |     |       |       |
| Yes                             | 2(18.18)          | 14(36.84)  | 0.256 | 0.025 | 2.607 | 0.25 |
| No                              | 9(81.82)          | 24(63.16)  |       |       |       |     |
| Transfusion                     |                   |             |     |       |       |
| Yes                             | 0(0)              | 2(5.26)    | 0    | 0     | -     | 0.999|
| No                              | 11(100)           | 36(94.74)  |       |       |       |     |
| Ingestion Undercook Meat        |                   |             |     |       |       |
| Yes                             | 3(27.27)          | 9(23.68)   | 0.566 | 0.08  | 3.989 | 0.568|
| No                              | 8(72.72)          | 29(76.32)  |       |       |       |     |
| Keeping Cat                     |                   |             |     |       |       |
| Yes                             | 1(9.1)            | 1(2.63)    | 8.327 | 0.32  | 216.43 | 0.202|
| No                              | 10(90.9)          | 37(97.37)  |       |       | 3      |     |
| Occupational Contact With Soil  |                   |             |     |       |       |
| Yes                             | 6(54.55)          | 6(15.79)   | 7.243 | 1.062 | 49.378 | 0.043*|
| No                              | 5(45.45)          | 32(84.21)  |       |       |       |     |
| Eating Unpasteurized Dairy Products |             |             |     |       |       |
| Yes                             | 2(18.18)          | 8(21.05)   | 2.256 | 0.255 | 19.932 | 0.464|
| No                              | 9(81.82)          | 30(78.95)  |       |       |       |     |

* Statistical significance was defined as a P-value of <0.05.