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ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

Toxoplasma gondii seroprevalence and related risk factors in patients with HIV in Iran

OBJECTIVE To determinate the seroprevalence of *Toxoplasma gondii* among patients with HIV, and other probable risk factors associated with this situation, including demographic and clinical factors. **METHOD** This retrospective study was carried out on 246 HIV positive patients at the Shiraz HIV/AIDS research center, in Southwest Iran, in the two years beginning March 2012. Seropositivity for HIV was diagnosed initially by the enzyme linked immunosorbent assay (ELISA) and subsequently confirmed by the Western Blot test. Detection of IgG antibodies against *T. gondii* was performed using an ELISA kit in accordance with the manufacturer's instructions. **RESULTS** Of 246 patients with HIV, the seroprevalence of anti-*T. gondii* IgG antibody was 20.7% (51 cases). The mean CD4+ lymphocyte count among seropositive patients was lower than that of the seronegative group, 335 ± 213 vs 390 ± 258 cells/mm³, respectively. The frequency of seropositive cases with a CD4+ lymphocyte count $<200/\mu\text{L}$ cells/mm³ was higher than that of seronegative cases, 27.5 vs 24.6%, respectively. **CONCLUSIONS** These findings highlight the importance of serological tests to investigate the consequences of *T. gondii* infection among patients with HIV, in addition to the CD4+ lymphocyte count, which alone is not a criterion of infection.

Toxoplasmosis is one of the most prevalent parasitic diseases in humans. It is caused by *Toxoplasma gondii* which is an obligate intracellular zoonotic protozoan with global distribution.¹ Humans, as the intermediate host, acquire *T. gondii* infection in different ways, mainly via oral ingestion of contaminated food or water, organ transplantation, blood transfusion or vertical transmission from the infected mother to her child.^{2,3} *T. gondii* is the most prevalent parasitic infection in humans, and it is estimated that approximately half of the world's population is infected, although most of the cases are asymptomatic.⁴ There is no national surveillance

on the seroprevalence of *T. gondii* in Iran, but regional studies indicate that about half of the Iranian people are seropositive.⁵

Most people infected with *T. gondii* remain asymptomatic because the immune system usually keeps the infection dormant,⁶ but in immunocompromised individuals with low levels of CD4+ lymphocytes the infection can cause severe disease.⁷ Although, based on the Iranian official records, less than 25,000 cases of human immunodeficiency virus (HIV) infection have been identified in the population, estimates from the Joint United Nations Programme on HIV/AIDS

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Περίληψη στο τέλος του άρθρου

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(UNAIDS) suggest that more than twice this number of HIV seropositive people are living in Iran.⁸ Among patients with HIV, cerebral toxoplasmosis has become one of the most frequent opportunistic infections and is the most common cause of focal lesions of the brain.^{7,9} Toxoplasmic encephalitis (TE) is a serious condition that can be fatal if not attended quickly.⁴

Serological testing to determine the presence of *T. gondii* in HIV patients is critical, since an early diagnosis can contribute to the application of appropriate treatment and prevention of the related complications.⁷ The findings of a regional study, such as that reported here, not only illustrate the situation in the region studied, but also provide the epidemiological background for future studies. The present study aimed to determine the seroprevalence of *T. gondii* among patients with HIV and other probable risk factors associated with this situation, including demographic and clinical factors, in Southwest Iran.

MATERIAL AND METHOD

Study design, setting, and population

This retrospective study was carried out during the two years beginning March 2012 at Shiraz HIV/AIDS Research Center, affiliated with the Shiraz University of Medical Sciences, located in Southwest Iran. The study participants consisted of 246 HIV positive patients with an active profile in the database of the research center. These patients had been tested for IgG antibodies against *T. gondii* within the study period. The only excluding criterion was lack of medical records. This study was conducted in accordance with the declaration of Helsinki; however, because only medical records were used and the identification details were kept strictly confidential and the analysis did not further affect the patients, the need for informed consent was waived.

Detection of HIV and antibodies against *T. gondii*

HIV seropositivity was diagnosed initially by the enzyme linked immunosorbent assay (ELISA) (Dia.Pro Diagnostic Bioprobes, Italy), and subsequently the primary positive ELISA results were confirmed by a Western Blot test. Detection of IgG antibodies against *T. gondii* was performed using an ELISA kit (EUROIMMUN™) in accordance with the manufacturer's instructions. A titer of anti-*T. gondii* IgG greater than 11 IU/mL was considered as positive, a value less than 9 IU/mL as negative, and the borderline titers were counted as negative. Demographic and clinical information, including age, gender, prison history, addiction history, transmission route, highly active antiretroviral therapy (HAART) status and CD4+ lymphocyte count were retrieved from the medical records under strict anonymity.

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS™) software (IBM Corp, USA), version 19.0. The results are presented as descriptive statistics in terms of relative frequency. Chi-square or Fisher's exact tests was used to estimate any statistical association for quantitative variables and *t*-tests were used to compare the means. Statistical significance was regarded at *p* values <0.05.

RESULTS

In the 246 patients with HIV studied, the seroprevalence of anti-*T. gondii* IgG antibody was 20.7% (51 cases). The majority (86.3%) of the seropositive results were found in the age range of 20–50 years. Although seropositivity for *T. gondii* was more common among the males, the gender differences compared to seronegative patients were not significant (tab. 1). A history of drug addiction and a prison record were more frequent among seropositive cases, although compared to seronegative cases, no statistical difference was detected. Intravenous drug use (IDU) and sexual contact were the most frequently reported routes for transmission of HIV infection among both groups, and no significant risk was demonstrated for any one specific transmission route for anti-*T. gondii* seropositivity.

Most of the participants in both groups had undergone HAART, and no association was found between HAART and anti-*T. gondii* seropositivity. Among the seropositive patients, 10 (31.3%) had undergone HAART before being tested for anti-*T. gondii* and 22 (68.7%) had undergone HAART after serological testing. For seropositive individuals who had undergone HAART either before or after serological testing for *T. gondii*, the main starting anti-retroviral drugs were almost the same, i.e. zidovudine, lamivudine and efavirenz. Among the seronegative cases, also, 32.3% and 67.7% underwent HAART before and after serological testing, respectively, and compared with seropositive cases, no significant difference was demonstrated.

The mean CD4+ lymphocyte count of the seropositive patients was lower than that of the seronegative group (335±213 vs 390±258 cells/mm³, respectively). The frequency of seropositive cases with CD4+ lymphocyte count <200/μL cells/mm³ was higher than that of the seronegative cases (27.5 vs 24.6%, respectively), but none of these differences was statistically significant. Finally, point by point CD4+ lymphocyte count analysis showed that with a decrease in the level of CD4+ lymphocyte below 500/μL cells/mm³ seropositivity for anti-*T. gondii* IgG became more likely (fig. 1).

Table 1. Association of demographic and clinical variables with *Toxoplasma gondii* infection in patients with HIV (n=246).

Studied factors	Groups	Seronegative no (%) Total no 195	Seropositive no (%) Total no 51	Significance level (p value)
<i>Age in years</i>				
Mean±SD		35±11	38±10	0.07
Range		2–64	24–70	
<19 years		14 (7.2)	0	
20–50		162 (83.1)	44 (86.3)	0.11
>51		19 (9.7)	7 (13.7)	
<i>Gender</i>				
Male		116 (59.5)	33 (64.7)	0.52
Female		79 (40.5)	18 (35.3)	
<i>HAART therapy</i>				
Yes		124 (63.6)	32 (62.7)	0.99
No		71 (36.4)	19 (37.3)	
<i>History of addiction</i>				
Yes		107 (54.9)	33 (64.7)	0.26
No		88 (45.1)	18 (35.3)	
<i>Prison history</i>				
Yes		93 (47.7)	27 (52.9)	0.53
No		102 (52.3)	24 (47.1)	
<i>Blood transfusion history</i>				
Yes		9 (4.6)	4 (7.8)	0.47
No		186 (95.4)	47 (92.2)	
<i>Transmission route</i>				
Intravenous drug use		95 (48.7)	29 (56.9)	0.3
Sexual contact		71 (36.4)	20 (39.2)	0.71
Mother to infant		13 (6.7)	0	ND*
Blood transfusion		2 (1)	0	ND
Occupational exposure		0	0	ND
Unknown		14 (7.2)	2 (3.9)	ND
<i>CD4+lymphocyte count</i>				
Mean±SD		390±258	335±213	0.16
Range		10–1,250	25–878	
<200/μL cells/mm ³		48 (24.6)	14 (27.5)	0.71
≥200/μL cells/mm ³		147 (75.4)	37 (72.5)	

HAART: Highly active antiretroviral therapy

*ND: Not determined, due to lack of the test assumptions

DISCUSSION

The seroprevalence of latent toxoplasma infection in patients with HIV, based on the detection of anti-*T. gondii* antibodies, has been documented to vary depending on several environmental and health factors within the studied

communities, and even the time.⁷ In the present study, the seroprevalence of latent toxoplasmosis in a series of patients with HIV was estimated at 20.7%, which is lower than in similar reports from other parts of Iran.

It is recognized that climatic changes, as an environmental risk factor, can affect the nature of *T. gondii* infection;¹⁰ Iran is a vast country with great climatic variability. The northern cities of the country, which experience a humid temperate climate, have reported a higher seroprevalence of anti-*T. gondii* IgG among HIV patients, 77.4% and 49.7% from Mazandaran and Tehran provinces, respectively,^{5,11} while a lower frequency (38.01%) was reported from the northeast of Iran (Mashhad city) where the weather is cold.¹² Closer to the findings of the present study, Davarpanah and colleagues, in a study over the two year from 2003, reported the lowest seroprevalence of toxoplasmosis (18.2%) among HIV patients in Iran from the same region (Shiraz city) which is characterized by dry weather.⁹ Variability in seroprevalence of latent toxoplasmosis has been reported in other parts of the world, for example, Ethiopia 87.4%, Brazil 80%, Nigeria 37.8%, and Korea up to 30%.^{7,13–15}

Of the several factors that can affect the seroprevalence of toxoplasmosis, one of the important is the food carrying the protozoan.¹⁶ Published data indicate low *Toxoplasma* seropositivity among farm animals in the province where the study was conducted.^{17–19} Blood transfusion, as a route of infection with *T. gondii*, was not a significant risk among the study patients, because of the low prevalence of *T. gondii* infection in blood products in the region, as shown by Shaddel and colleagues,²⁰ and no vertical transmission from mother to infant was found definitively in this series. While there are no recent data on the seroprevalence of *T. gondii* antibodies among pregnant women in the study region, low risk for infection via mother was indicated by Asgari and colleagues, who documented a prevalence of 14.4% *T. gondii* among spontaneously aborted fetuses.²¹

Depletion of CD4+ lymphocytes has been noted as an important risk factor for reactivation of latent infection of *T. gondii* among HIV patients.³ In this series, although the mean CD4+ lymphocyte count was lower among the seropositive than in the seronegative cases for anti-*T. gondii* IgG, the difference was not significant. Development of toxoplasmosis or other opportunistic infections among HIV patients with a high level of CD4+ lymphocytes is not uncommon,²² and the level of CD4+ lymphocytes is not always a reliable indicator for presumption of opportunistic infections such as toxoplasmosis, while the presence of anti-*T. gondii* IgG is more likely to represent a latent infection.^{3,5}

In summary, apart from the epidemiological findings and

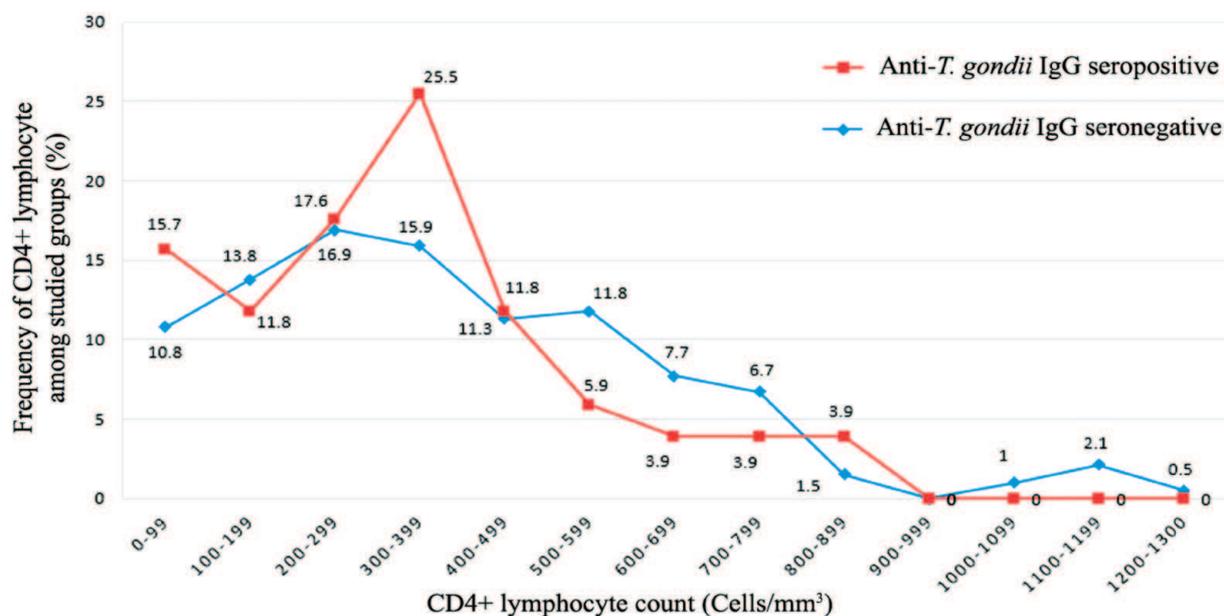


Figure 1. The distribution pattern of CD4+ lymphocyte count in patients with HIV (n=246) according to seropositivity for anti-*Toxoplasma gondii* IgG.

the associated risk factors in the studied area, the study results highlight the importance of serological testing to confirm *T. gondii* infection among patients with HIV and deal with the consequences, in addition to the level of CD4+ lymphocytes, which alone is not always a reliable criterion of infection.

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ΠΕΡΙΛΗΨΗ

Οροθετικότητα λανθάνουσας τοξοπλάσμωσης και σχετιζόμενοι παράγοντες κινδύνου σε HIV θετικούς ασθενείς στο Ιράν

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ΣΚΟΠΟΣ Καθορισμός της οροθετικότητας του *T. gondii* σε ασθενείς με HIV, καθώς και άλλων πιθανών παραγόντων κινδύνου που σχετίζονται, περιλαμβανομένων δημογραφικών και κλινικών παραγόντων. **ΥΛΙΚΟ-ΜΕΘΟΔΟΣ** Η εν λόγω αναδρομική μελέτη διεξήχθη σε 246 θετικούς στον HIV ασθενείς, στο Κέντρο Έρευνας Shiraz HIV/AIDS στο νοτιοδυτικό Ιράν εντός μιας διετίας, αρχόμενης από τον Μάρτιο του 2012. Οι HIV οροθετικοί ασθενείς διαγνώστηκαν αρχικά με ELISA και επιβεβαιώθηκαν με δοκιμασία Western Blot. Η ανίχνευση των IgG αντισωμάτων κατά του *T. gondii* πραγματοποιήθηκε με τη μέθοδο ELISA. **ΑΠΟΤΕΛΕΣΜΑΤΑ** Τα αποτελέσματα έδειξαν ότι από τους 246 HIV θετικούς ασθενείς που μελετήθηκαν, το 20,7% (51 ασθενείς) είχε οροθετικότητα για IgG αντι-*T. gondii* αντισώματα. Ο μέσος αριθμός των CD4+ λεμφοκυττάρων στους οροθετικούς ασθενείς ήταν χαμηλότερος σε σχέση με την ομάδα των οροαρνητικών (335±213 έναντι 390±258 cells/μL, αντίστοιχα). Επίσης, οι οροθετικοί με αριθμό CD4+ λεμφοκυττάρων <200 cells/μL ήταν περισσότεροι από τους οροαρνητικούς (27,5% έναντι 24,6%, αντίστοιχα). **ΣΥΜΠΕΡΑΣΜΑΤΑ** Τα σχετι-

κά ευρήματα τόνισαν τη σημασία των ορολογικών δοκιμασιών σχετικά με τη διερεύνηση των συνεπειών της λοίμωξης από *T. gondii* των HIV θετικών ασθενών, εκτός από το επίπεδο των CD4+ λεμφοκυττάρων, αφού αυτά δεν αποτελούν από μόνα τους κριτήριο λοίμωξης.

Λέξεις ευρητηρίου: CD4+ λεμφοκύτταρα, HIV/AIDS, Ιράν, *Toxoplasma gondii*, Τοξοπλάσμωση

References

- HALONEN SK, WEISS LM. Toxoplasmosis. *Handb Clin Neurol* 2013, 114:125–145
- OSUNKALU VO, AKANMU SA, OFOMAH NJ, ONYIAORAH IV, ADEDIRAN AA, AKINDE RO ET AL. Seroprevalence of *Toxoplasma gondii* IgG antibody in HIV-infected patients at the Lagos University Teaching Hospital. *HIV AIDS (Auckl)* 2011, 3:101–105
- WALKER M, ZUNT JR. Parasitic central nervous system infections in immunocompromised hosts. *Clin Infect Dis* 2005, 40:1005–1015
- NISSAPATORN V, LEE C, QUEK KF, LEONG CL, MAHMUD R, ABDULLAH KA. Toxoplasmosis in HIV/AIDS patients: A current situation. *Jpn J Infect Dis* 2004, 57:160–165
- DARYANI A, SHARIF M, MEIGOUNI M. Seroprevalence of IgG and IgM anti-Toxoplasma antibodies in HIV/AIDS patients, northern Iran. *Asian Pac J Trop Med* 2011, 4:271–274
- HO YC, SUN HY, CHEN MY, HSIEH SM, SHENG WH, CHANG SC. Clinical presentation and outcome of toxoplasmic encephalitis in patients with human immunodeficiency virus type 1 infection. *J Microbiol Immunol Infect* 2008, 41:386–392
- 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, 55:25–30
- ZADEH AO, SEYEDALINAGHI S, HASSANZAD FF, HAJIZADEH M, MOHAMADI S, EMAMZADEH-FARD S ET AL. Prevalence of HIV infection and the correlates among homeless in Tehran, Iran. *Asian Pac J Trop Biomed* 2014, 4:65–68
- DAVARPANAH MA, MEHRABANI D, NEIRAMI R, GHAREMANPOORI M, DARVISHI M. Toxoplasmosis in HIV/AIDS patients in Shiraz, southern Iran. *Iran Red Crescent Med J* 2007, 9:22–27
- MEERBURG BG, KIJLSTRA A. Changing climate-changing pathogens: *Toxoplasma gondii* in North-Western Europe. *Parasitol Res* 2009, 105:17–24
- MOHRAZ M, MEHRKHANIF, JAM S, SEYEDALINAGHI S, SABZVARI D, FATTAHI F ET AL. Seroprevalence of toxoplasmosis in HIV(+)/AIDS patients in Iran. *Acta Med Iran* 2011, 49:213–218
- SHAFIEI R, RIAZI Z, SARVGHAD M, GALIAN SHARIFDINI M, MAHMOODZADEH A, HAJIA M. Prevalence of IgG and IgM anti-*Toxoplasma gondii* antibodies in HIV positive patients in Northeast of Iran. *Iran J Pathol* 2011, 6:68–72
- WALLE F, KEBEDE N, TSEGAYE A, KASSA T. Seroprevalence and risk factors for toxoplasmosis in HIV infected and non-infected individuals in Bahir Dar, Northwest Ethiopia. *Parasit Vectors* 2013, 6:15
- OGOINA D, ONYEMELUKWE GC, MUSA BO, OBIAKO RO. Seroprevalence of IgM and IgG antibodies to *Toxoplasma* infection in healthy and HIV-positive adults from Northern Nigeria. *J Infect Dev Ctries* 2013, 7:398–403
- YANG Z, CHO PY, AHN SK, AHN HJ, KIM TS, CHONG CK ET AL. A surge in the seroprevalence of toxoplasmosis among the residents of islands in Gangwha-gun, Incheon, Korea. *Korean J Parasitol* 2012, 50:191–197
- TENTER AM, HECKEROTH AR, WEISS LM. *Toxoplasma gondii*: From animals to humans. *Int J Parasitol* 2000, 30:1217–1258
- SARKARI B, ASGARI Q, BAGHERIAN N, ASHKANI ESFAHANI S, KALANTARI M, MOHAMMADPOUR I ET AL. Molecular and serological evaluation of *Toxoplasma gondii* infection in reared turkeys in Fars Province, Iran. *Jundishapur J Microbiol* 2014, 7:e11598
- ASGARI Q, FARZANEH A, KALANTARI M, AKRAMI MOHAJERI F, MOAZENI M, ZARIFI M ET AL. Seroprevalence of free-ranging chicken toxoplasmosis in sub-urban regions of Shiraz, Iran. *Int J Poult Sci* 2006, 5:262–264
- ASGARI Q, SARKARI B, AMERINIA M, PANAH S, MOHAMMADPOUR I, SADEGHI SARVESTANI A. *Toxoplasma* infection in farm animals: A seroepidemiological survey in Fars Province, South of Iran. *Jundishapur J Microbiol* 2013, 6:269–272
- SHADDEL M, MIRZAII-DIZGAH I, HOSHANGI M. Anti-*Toxoplasma gondii* antibody levels in blood supply of Shiraz Blood Transfusion Institute, Iran. *Iran J Parasitol* 2014, 9:120–124
- ASGARI Q, FEKRI M, MONABATI A, KALANTARY M, MOHAMMADPOUR I, MOTAZEDIAN MH ET AL. Molecular genotyping of *Toxoplasma gondii* in human spontaneous aborted fetuses in Shiraz, Southern Iran. *Iran J Public Health* 2013, 42:620–625
- HOFFMANN C, ERNST M, MEYER P, WOLF E, ROSENKRANZ T, PLETTERBERG A ET AL. Evolving characteristics of toxoplasmosis in patients infected with human immunodeficiency virus-1: Clinical course and *Toxoplasma gondii*-specific immune responses. *Clin Microbiol Infect* 2007, 13:510–515

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