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Serological Study of *Toxoplasma gondii* Infection Using IFA Method in Renal Transplant Recipients

*M Nateghi Rostami [1], E Eskandari [2], Z Garoosi [1], N Mohajeri [1], M Rezaian [1], *H Keshavarz [1]*

[1]*Dept. of Medical Parasitology and Mycology, School of Public Health & Institute of Public Health Research, Tehran University of Medical Sciences, Iran*
[2]*Center for Research and Training in Skin Diseases and Leprosy, Tehran University of Medical Sciences, Iran*

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Abstract

Toxoplasmosis is a wide distributed opportunistic infection caused by *Toxoplasma gondii*. This was a cross-sectional study of *T. gondii* antibody titer, which was conducted from June 2003 to August 2004 on renal transplant recipients in Iran. A total of 551 serum samples were obtained from randomly selected population referred from different areas all over the country to Shafa Central Clinic in Tehran. Patient's information was recorded in a questionnaire before sampling. Two samples of finger-prick blood were collected from each person and antibody titer against *Toxoplasma* was assessed by Indirect Fluorescence Antibody (IFA) technique on serum samples. Totally 39 cases (7.1%) of samples were positive for antibody by the titer of 1: 20 and higher. On investigation of risk factors, no significant difference was found between consumption of under-cooked meat, close contact with animals, and the source of drinking water and seropositivity rate of toxoplasmosis. The relatively low seroprevalence rate of *Toxoplasma* infection shows the successful approaches to awareness of transplant recipients about the potential risks of acquisition of infectious diseases due to regular administration of suppressive drugs. However, the regular surveillance through serological screening of *Toxoplasma* antibody in kidney transplant recipients is advisable.

Keywords: *Toxoplasmosis*, *Renal transplant recipients, Serology, Iran*

Introduction

Toxoplasma gondii is a coccidian parasite of the phylum Apicomplexa that can infect humans and a broad spectrum of warm-blooded animals serving as intermediate hosts (1). Toxoplasmosis is a wide distributed opportunistic infection around the world, which might be influenced by factors of human behavior, sanitary situation, and climate conditions (2). Although toxoplasmosis generally is asymptomatic in otherwise healthy individuals, its devastating manifestations in im munocompromised individuals, including patients receiving immunosuppressive therapy with corticosteroids, accentuate the need for monitoring and management of toxoplasmosis in these patients (3). Because of the continuous administration of immuno-suppressive drugs among renal transplant recipients, they are prone to acquire many opportunistic parasite infections; one of the most common organisms among them is *T. gondii* (4). Diagnosis of toxoplasmosis in humans is usually made by serological, histological, and molecular methods, or by some combination of the above (5). The use of serologic tests to show specific antibody to *T. gondii* is the primary and the most common approach to diagnosis. There are numerous serological procedures avail-

***Corresponding author:** Tel: +98 21 88951392,
E-mail: hkeshavarz@tums.ac.ir

able for the detection of specific antibodies to *Toxoplasma* such as IFA (6).
The objective of this study was to determine the seropositivity rate of *Toxoplasma* antibody in the renal transplant recipients using IFA method.

Materials and Methods

Study population
This was a cross-sectional study, which was conducted at the laboratory of serology of protozoa, School of Public Health, Tehran University of Medical Sciences, from June 2003 to August 2004. The people included in this study were residents of different areas all over Iran. A total of 551 serum samples were obtained from randomly selected patients referred to the Shafa Central Clinic for renal transplant patients, in Tehran. Each case was asked to fill out a questionnaire including demographic and baseline data and socioeconomic conditions.
Laboratory methods
Two samples of finger-prick blood were collected from each person in heparinized microhematocrit tubes. Serum samples were stored at -20 ºC until being examined. The antibody titer against *Toxoplasma* was assessed by IFA technique using Fluorescein labeled anti-human globulin (AHG*). Serum dilutions of 1: 20, 1: 100, and 1:200 were prepared from each sample to be tested. If positive, further serial two-fold dilutions were tested. All prepared slides were examined under a Zeiss fluorescent microscope. Titers of 1: 20 and above were considered as positive (7).
Statistical methods
Data were analyzed using SPSS (version 12) software. Significance of difference was analyzed by chi-squared and Fisher's exact tests. Odds ratio was calculated whenever it needed. $P< 0.05$ was considered significant.

Results

Totally 39 cases (7.1%) were positive for specific antibody by the titer of 1: 20 and above (Table 1). The difference between female and male subjects was not statistically significant (Table 2). The odds ratio of being male to female in acquiring the *Toxoplasma* infection was not different in a confidence interval of 95%.
There was no significant difference between seropositive subjects with and without closed contact with domestic animals (Table 2).
Analysis of water source revealed that there was not significant difference between individuals who had pipeline water source and who had unfiltered water in seropositivity rate (Table 2).
People who consumed under cooked meat showed no significant difference with people who did not, in rate of *Toxoplasma* antibody development (Table 2).
The effect of previous chronic diseases (such as blood pressure and diabetes), the influence of educational level, and the type of house (apartment or villa) of the population under study on seropositivity rate of *Toxoplasma* antibody have also been studied. No seropositivity significant relationship was found with these factors.
The age distribution of population under study according to *Toxoplasma* seropositivity is outlined in Fig. 1. Accordingly, the same trend as general non-infected population is observed in seropositive patients, so that the higher positive titer of antibody could be seen with patients in the age range of 35-50 yr (middle-aged) ($P< 0.05$).

Table 1: Distribution of *Toxoplasma* antibody titers in renal transplant recipients using IFA method in 2003-2004*

Antibody Titer	Frequency	
	Number	percent
<1:20 (Neg)	512	92.9
1:20	18	3.3
1:100	15	2.7
1:200	6	1.1
Total	551	100

* Titer of 1:20 and above regarded as probability of *Toxoplasma gondii* infection

Table 2: Seroprevalence of toxoplasmosis by baseline characteristics and possible risk factors in renal transplant recipients using IFA method*

Characteristics/ Risk factors	*Toxoplasma* seropositivity			*P* value†	Odds ratio
	Total n	Positive n (%)	Negative n (%)		
Gender					
-Male	325	25 (7.7)	300 (92.3)	0.5	1.24
-Female	226	14 (6.2)	212 (93.8)		(95% CI: 0.66-2.3)
Animal contact					
-Yes	71	4 (5.6)	67 (94.4)	0.61	0.76
-No	480	35 (7.3)	445 (92.7)		(95% CI: 0.26-2.2)
Water source					
-Pipeline	549	39 (7.1)	510 (92.9)	0.69	
-Unfiltered	2	0 (0.0)	2 (100)		
Taking undercooked meat					
- Yes	23	2 (8.7)	21 (91.3)		1.26
- No	528	37 (7.0)	491 (93.0)	0.76	(95% CI: 0.29-5.6)
Chronic disease					
- Yes	423	33 (7.8)	390 (92.2)	0.23	1.7
- No	128	6 (4.7)	122 (95.3)		(95% CI: 0.7-4.2)
Education					
- Illiterate	101	7 (6.9)	94 (93.1)	0.6	
- Primary	175	14 (8.0)	161 (92.0)		
- Secondary	93	7 (7.5)	86 (92.5)		
- High	123	5 (4.1)	118 (95.9)		
- University	59	6 (10.2)	53 (89.8)		
House					
- Apartment	262	20 (7.6)	242 (92.4)	0.62	0.85
- Villa	289	19 (6.6)	270 (93.4)		(95% CI: 0.44-1.6)
Total	551	39 (7.1)	512 (92.9)		

* Titer of 1:20 and above regarded as probability of *Toxoplasma gondii* infection
† Results of $\chi 2$ test and Fisher's exact test by *P* value of <0.05 as significant difference

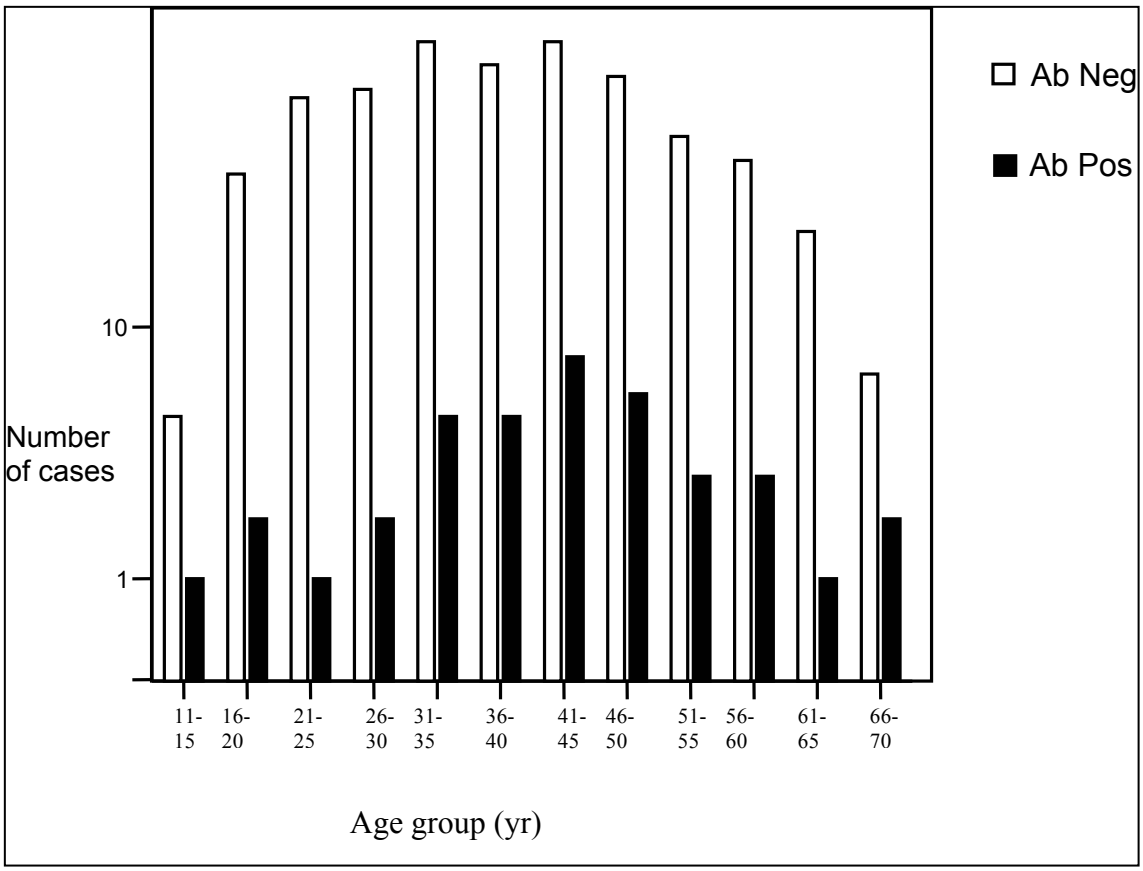

Fig. 1: Age distributions of population contribute to the serologic study of *Toxoplasma* infection in renal transplant recipients (titer of 1:20 and above regarded as probability of *Toxoplasma gondii* infection)

Discussion

Toxoplasmosis is a well-known opportunistic infection of worldwide distribution in immunocompromised patients. *T. gondii* infection regularly triggers specific antibody development, which varies considerably among different geographic areas and among individuals within a given population. The use of serologic tests to show specific antibody to *T. gondii* is the primary method of diagnosis. IgG antibodies usually appear within 1 to 2 weeks of acquisition of the infection, peak within 1 to 2 mo, decrease at variable rates, and usually persist for life (8).

Several epidemiological studies reported a broad range of serologic prevalence of *Toxoplasma* antibody titers among residents of different localities of Iran (9-13). According to Assmar *et al*. (9) a total of 51.8% of more than 13000 serum samples collected from 12 provinces in Iran showed positive *Toxoplasma* antibody using IFA method. This is ranged from 20.5% in Mazandaran Province, northern part to 2.9% in Hormozgan Province, southern part of Iran. The overall seropositive rate of 12.8% was found in north-west and south-west parts of Iran (12). Study of Ghorbani *et al*. (11) in Caspian Sea area revealed that as high as 55.7% of sera were positive with titers of 1:20 or above. Totally, 7.1% of population under this study was positive for antibody by the titer of 1: 20 and higher. Low sero-positive rate

in our study may be, partially, due to administration of immunosuuresive drugs in the parasites. In the work of Sukthana *et al.* (32), *T. gondii* IgG antibodies were determined in 200 kidney recipients by the Sabin-Feldman dye test and twenty-two (11%) cases were positive for antibody detection. In a previous limited study on kidney recipients in Iran sum of 91% of 64 patients were positive for *Toxoplasma* antibody using IFA and PCR methods (14).
Our findings revealed that the age pattern is consistent with the highest exposure to infection in the middle-aged population. A relatively similar age-dependent distribution of *Toxoplasma* antibody is indicated in the work of Assmar *et al*. (9). The increasing trend in seropositivity is a usual finding, which has been reported in several studies (12, 15, 16).
In accordance with sex differences, the prevalence was found to be higher in some studies in females (12, 15), and sometimes in males (17), however, consistent with some studies (9, 11, 16) we found no sex difference in seropositivity rate.
Immunosuppressive patients are exposed to various possible risk factors, which might expose them to *Toxoplasma* primary infection or reactivation (18, 19). Although some investigators attribute great importance to the consumption of undercooked meat or contact with cats in high seropositivity of antibodies to *T. gondii*, no such significant association was found in our study regarding exposure to sources of infection (i.e. oocyst or tissue cyst) including consumption of under-cooked meat, close contact with domestic animals, and drinking of unfiltered water and seropositivity of toxoplasmosis. The lack of association between history of contact with animals and/or taking under-cooked meat and seropositivity was previously reported in some studies (13, 20). Nevertheless, on the other side are findings, which showed such association. In the study of Sukthana *et al*. (32), a statistically significant difference between sero-positive and sero-negative subjects occurred in the history of taking under-cooked meat. Similarly, a significant difference was obtained between seroprevalence and consumption of undercooked meat, contaminated vegetables, and contact with animal in renal transplant recipients in Iran (14).
Since infection in humans most commonly occurs through the ingestion of undercooked meat that contains tissue cysts and through the ingestion of water or food contaminated with oocysts, patients should well-informed on the risk of exposure to such infective materials. In European countries such as France, where eating undercooked meat is common and the prevalence of the infection is high, meat may be an important source of the infection (21- 24). In contrast are countries such as those in Africa and Central America, where the ingestion of contaminated food products probably accounts for infection (16, 25, 26).
T. gondii infection has been reported between 1 d and 13 mo after transplantation and reactivations have been described up to 7 years after transplantation (27-30). In Iran, renal transplant donors and recipients are not routinely screened for toxoplasmosis since *T. gondii* infections in renal transplant recipients are thought to be rare.
In the present study, in comparison with other studies, the low prevalence rate of infection might show the successful approaches to awareness of transplant recipients about the potential risks of acquisition of infectious diseases due to regular administration of suppressive drugs. In addition, the style of life and socioeconomic conditions of the studied population, like urbanization, should influence the rate of antibody development against *Toxoplasma* (2). Health system managers should con-

tinue to offer education that help prevention of infectious disease as toxoplasmosis in immunosuppressed patients. Improving the level of knowledge about toxoplasmosis and relevant risk factors would have obvious influence on the withdrawing the infection rate amongst this population.

Even though apparently the seropositivity rate of infection in this group of patients is under control, the regular surveillance through serological screening of *Toxoplasma* antibody in kidney transplant recipients is advisable. In addition, all seronegative women should well inform on the risks of exposure to *T. gondii* infection (1, 31).

We expected the studied transplant recipients be more vulnerable to *Toxoplasma* infection due to regular immunosuppressive drug administration. Present study on this population who are originated from all over the country showed acceptable rate of *Toxoplasma* infection using IFA. Because of useful permanent education, none of risk factors showed significant involvement in the seropositivity rate.

Taken as a whole, the low prevalence rate of infection might show the successful approaches to awareness of transplant recipients about the potential risks of Toxoplasmosis, although, the regular surveillance through serological screening of *Toxoplasma* antibody is advisable.

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References

1. Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet. 2004; 363 (9425): 1965-76.
2. Macpherson CN. Human behaviour and the epidemiology of parasitic zoonoses. Int J Parasitol. 2005; 35: 1319-31.
3. Montoya JG, Kovacs JA, Remington JS. *Toxoplasma gondii*. In: Principles and practice of infectious diseases. Mandell GL, Bennett JE, Dolin R, EdsitorsNew York: Churchill Livingstone, 2000; pp: 3170-98.
4. Ambroise-Thomas P. Parasitic diseases and immunodeficiencies. Parasitology. 2001; 122: S65-S71.
5. Montoya JG. Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. J Infec Dis. 2002; 185: S73-S82.
6. Remington JS, Thulliez P, Montoya JG. Recent developments for diagnosis of toxoplasmosis. J Clin Microbiol. 2004; 42(3): 941–945.
7. Kelen AE, Ayllon-Leindl L, Labsoffsky NA. Indirect Fluorescent Antibody method in serodiagnosis of toxoplasmosis. Can J Microbiol. 1962; 8: 545.
8. Iqbal J, Nampoory MR, Johnv KV, *et al*. Determination of antibodies to *Toxoplasma gondii* and CMV in renal transplant recipients. Transplant Proc. 2003; 35 (7): 2703-5.
9. Assmar M, Amirkhani A, Piazak N, *et al*. Toxoplasmosis in Iran. Results of a seroepidemiological study. Bull Soc Pathol Exot. 1997; 90: 19-21.
10. Hoghooghi-Rad N, Afraa M. Prevalence of toxoplasmosis in humans and domestic animals in Ahwaz, capital of Khoozestan Province, southwest Iran. J Trop Med Hyg. 1993; 96(3): 163-8.
11. Ghorbani M, Edrissian GH, Assad N. Serological survey of toxoplasmosis in the northern part of Iran using IFA technique. Trans Roy Soc Trop Med Hyg. 1978; 72: 369.

12. Ghorbani M, Edrissian GH, Afshar A. Serological survey of human toxoplasmosis in mountainous regions of the north-west and south-west parts of Iran. Trans Roy Soc Trop Med Hyg. 1981; 75: 38.
13. Sedaghat A, Ardehali SM, Sadigh M *et al*. The prevalence of *Toxoplasma* infection in southern Iran. J Trop Med Hyg. 1978; 81: 204-7.
14. Gharavi MJ. Prevalence and diagnosis of toxoplasmosis in immunodeficient patients. Abstract book of 2nd National Congress of Parasitic Diseases, Tehran University of Medical Sciences, Iran; 1997.
15. Abdel-Hameed AA. Sero-epidemiology of toxoplasmosis in Gezira, Sudan. J Trop Med Hyg. 1991; 94: 329-332.
16. Sobral CA, Amendoeira MR, Teva A, *et al*. Seroprevalence of infection with *Toxoplasma gondii* in indigenous Brazilian populations. Am J Trop Med Hyg. 2005; 72(1): 37-41.
17. Excler JL, Pretat E, Pozzetto OB, *et al*. Ser-epidemiological survey of toxoplasmosis in Burundi. Tropenmedizin and Parasitologie. 1988; 39: 139-141.
18. de Amorim Garcia CA, Orefice F, de Oliveira Lyra C *et al*. Socioeconomic conditions as determining factors in the prevalence of systemic and ocular toxoplasmosis in Northeastern Brazil. Ophthalmic Epidemiol. 2004; 11(4): 301-17.
19. Kortbeek LM, De Melker HE, Veldhuijzen IK *et al*. Population-based *Toxoplasma* seroprevalence study in the Netherlands. Epidemiol Infect. 2004; 132(5): 839-45.
20. Nissapatorn V, Noor Azmi MA, Cho SM *et al*. Toxoplasmosis: prevalence and risk factors. J Obs Gyn. 2003; 23: 618-24.
21. Huldt G, Lagercrantz R, Sheehe PR. On the epidemiology of human toxoplasmosis in Scandinavia especially in children. Acta Ped Scan. 1979; 68:745.
22. Dubey JP, Gamble HR, Hill D. High prevalence of viable *Toxoplasma gondii* infection in market weight pigs from a farm in Massachusetts. J Parasitol. 2002; 88: 1234-38.
23. Contreras M, Schenone H, Salinas P *et al*. Seroepidemiology of human toxoplasmosis in Chile. Rev Inst Med Trop Sao Paulo. 1996; 38(6): 431-5.
24. Dias RA, Navarro IT, Ruffolo BB, *et al*. *Toxoplasma gondii* in fresh pork sausage and seroprevalence in butchers from factories in Londrina, Parana State, Brazil. Rev Inst Med Trop Sao Paulo. 2005; 47(4): 185-9.
25. Frenkel JK, Ruiz A. Human toxoplasmosis and cat contact in Costa Rica. Am J Trop Med Hyg. 1980; 29: 1167.
26. Ahmed HJ, Mohammed HH, Yusuf MW, *et al*. Human toxoplasmosis in Somalia. Trans Roy Soc Trop Med Hyg. 1988; 82: 330-32.
27. Aubert D, Foudrinier F, Villena I, *et al*. PCR for diagnosis and follow-up of two cases of disseminated toxoplasmosis after kidney grafting. J Clin Microbiol. 1996; 34: 1347.
28. Giordano LFCM, Lasmar EP, Tavora ERF *et al*. Toxoplasmosis transmitted via kidney allograft: case report and review. Transplant Proc. 2002; 34:498–499.
29. Renoult E, Georges E, Biava MF *et al*. Toxoplasmosis in kidney transplant recipients: report of six cases and review. Clin Infect Dis. 1997; 24: 625–634.

30. Wulf MWH, Van Crevel R, Portier R *et al*. Toxoplasmosis after renal transplantation: Implications of a missed diagnosis. J Clin Microbiol. 2005; 43: 3544-47.
31. Dubey JP. Strategies to reduce transmission of *Toxoplasma gondii* to animals and humans. Vet Parasitol. 1996; 64 (1-2): 65-70.
32. Sukthana Y, Chintana T, Damrongkitchaiporn S *et al*. Serological study of *Toxoplasma gondii* in kidney recipients. J Med Assoc Thai. 2001; 84(8): 1137-41.