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Investigation of *Toxoplasma gondii* Infection in Cutaneous Leishmaniasis Patients of the Isfahan Province

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Abstract

Background: *Toxoplasma gondii*, the etiologic agent of toxoplasmosis, is the most prevalent protozoan parasite in the world.
Objectives: Due to high prevalence of toxoplasmosis as well zoonotic cutaneous leishmaniasis with *Leishmania major* origin in Isfahan, the main objective of this study was to determine, whether there is any correlation between these two protozoan infections and the intensity of the high prevalence of co-infection.
Methods: All the case and control samples (80 individuals), collected from suspected patients that had referred for a skin test to the Isfahan dermatology and Leishmaniasis research center from August to November 2014, were investigated. About 2 mL of venipuncture blood was taken from each case and control group member, and serum was separated and stored at -20°C until the enzyme linked immunosorbent assay (ELISA) for detection of anti-*Toxoplasma gondii* specific IgM and IgG antibodies.
Results: Overall, 27 (33.8%) candidates in the control group and 33 (41.3%) patients with Cutaneous Leishmaniasis (CL) were positive for Toxo-IgG. All samples were found to be Toxo-IgM negative. The prevalence difference of *Toxoplasma* infection in healthy controls and CL patients was not significant.
Conclusions: The results of the present study showed that the prevalence difference of *Toxoplasma* infection in healthy controls and CL patients was not significant. This means, there was no relationship between infection with CL and toxoplasmosis, thus infection with *Toxoplasma gondii* wasn't a risk factor for infection with Leishmaniasis; *Toxoplasma gondii* didn't show a preventive or inhibitory role against *Leishmania* infection.

Keywords: Cutaneous leishmaniasis, ELISA, Isfahan, *Toxoplasma gondii*

1. Background

Toxoplasma (T.) gondii is a member of Apicomplexa (1). It is an obligate intracellular parasite and proliferates inside host cells as well as macrophages (2). *Toxoplasma gondii* infects more than 60% of the population worldwide (3). It can infect almost all warm-blooded creatures such as, human, animals, birds and marine mammals (4). *Toxoplasma gondii* is able to pass both sexual and asexual parts of its life cycle in feline (cat) as the final host, and a wide spectrum of warm-blooded vertebrate hosts including humans as intermediate hosts (5). The parasites pass through the placenta, blood transfusions, infected needles, vegetables contaminated with oocysts and undercooked meat and its' products (6). Increased human contact with domestic animals and wildlife can raise *Toxoplasma* infections. Interestingly, due to infection in marine mammals, toxoplasmosis is a prevalent water-borne disease in humans around the world (7, 8). About 38 strains of *T. gondii* have been isolated from different hosts around the world, amongst them, 10 strains have been pathogenic with basically the same genotype (9). *Toxoplasma gondii* has three genetic lineages namely; type 1, 2 and 3, type 2 being more common in human infections (10). It can cause opportunistic infections in humans and animals, yet in immunocompetent individuals almost all infections are asymptomatic infections. *Toxoplasma gondii* is the third major cause of food-related deaths in the United States (11). Leishmaniasis caused by a protozoan parasite of the genus *Leishmania* belongs to the family of trypanosomatidae. It inoculates to the skin by the bite of infected Phlebotomine sand fly (12). More than 20 different species of the genus *Leishmania* are known pathogens for humans (13), and about 90% of CL in the world is caused by *Leishmania major*. Almost all cases occur in Afghanistan, Iran, Iraq, Sudiaarabia, Syria, Algeria, Brazil and Peru (14).

2. Objectives

The main objective of the present study was to determine whether there is any correlation between these two

protozoan infections and the intensity of the high prevalence of co-infection.

3. Methods

All the case and control samples (80 individuals) were collected from suspected patients that had referred to the Isfahan dermatology and Leishmaniasis research center from August to November 2014. About 2 mL of venipuncture blood was taken from each case and control group members, serum was separated and stored at -20°C until the enzyme linked immunosorbent assay (ELISA) tests for detecting the anti-*T. gondii* specific IgM and IgG antibodies. The anti-*Toxoplasma* specific IgM and IgG were assessed using ELISA kits (EUROIMMUN, Lubeck, Germany). The procedure was performed according to the manufacturer's protocol.

3.1. Statistical Methods

Logistic Regression and Chi square statistical methods for analysis were performed by the SPSS 16 software.

4. Results

Various statistics derived from the data are presented in Tables 1 - 3. Overall, 27 (33.8%) cases in the control group and 33 (41.3%) patients with CL were positive for Toxo-IgG. The seroprevalence of Toxo-IgG was not significantly different between the two case and control groups (P = 0.414). In Toxo-IgG positive cases, including 28 (35%) males and 32 (40%) females, there was no significant difference between different genders (P = 0.624). All subjects were found to be Toxo-IgM negative. The logistic regression analysis showed that there was no significant relationship between the IgG positivity (P = 0.638, OR = 0.868) and age (P = 0.678, OR = 0.997) and gender (P = 0.437, OR = 0.788) in the control group and CL patients. In this study, the mean age of Toxo-IgG positive patients was 33.13 ± 13 years and in case of Toxo-IgG negative, the mean age was 26.8 ± 10 years.

Table 1. The Correlation Between *Toxoplasma gondii* IgG and Gender in Patients with Cutaneous Leishmaniasis

	CL Patients		Total
	Positive (N)	Negative (N)	
Toxo*IgG*	33	47	80
Gender			
Male	15	25	40
Female	18	22	40

Table 2. The Correlation Between *Toxoplasma gondii* IgG and Gender in Healthy Individuals[a,b]

	Control Group		Total
	Positive (N)	Negative (N)	
Toxo*IgG*	27	53	80
Gender			
Male	13	27	40
Female	14	26	40

Abbreviation: N, number.
[a] $P \leq 0.05$ is significant.
[b] P value (Toxo*IgG*) = Chi square = 0.414; P value (Toxo*IgG*) = Logestic Regression = 0.638; P value (Gender) = Chi square = 0.624; P value (Gender) = Logestic Regression = 0.437.

5. Discussion

The average seroprevalence of toxoplasmosis is about 36% in Iran. It spreads with maximum and minimum seropositivity for serum anti *Toxoplasma* IgG with 70% in northern and 15% in southeastern regions of Iran, determined using the ELISA technique, respectively. This is about 45% in Isfahan city Iran (15). Isfahan has been the most important location for zoonotic CL in Iran. These highly prevalent toxoplasmosis and CL, suggest that there is a probable relationship between these two prevalent parasitic protozoan infections (16).

The present study was designed and conducted to assess any positive or negative probable relationship between the two current and important parasitic diseases in Isfahan.

There are many documents and reports on the interaction and co-infections of two or more parasitic infections in the literature for example, a study on co-infection of *T. gondii* and *Toxocara* spp. conducted by Jones, that found a positive correlation between *Toxocara* spp. and *T. gondii* seroprevalence (17). In another study it was shown that there is a significant direct relationship between the high prevalence of *Toxoplasma* infection and schizophrenia (18). There are some studies on *T. gondii* as a risk factor for some other infective and even non-infective diseases due to the opportunistic property of this parasite. In a study performed by Shirbazou et al. they investigated the role of *T. gondii* as a risk factor for diabetes due to presence of *toxoplasma* in pancreatic beta cells and their distraction. They found that the prevalence of anti-*Toxoplasma* IgG antibodies in people with diabetes is about 2 times higher compared to healthy controls (19). Mohraz showed the high risk of toxoplasmic encephalitis in patients with Acquired Immunodeficiency Syndrome (AIDS) due to co-infection of *T. gondii* and Human Immunodeficiency Virus (HIV) in

Table 3. Correlation Between *Toxoplasma gondii* IgG Infection and Age Group in Healthy Individuals and Patients with Cutaneous Leishmaniasis[a,b]

Age Group	CL Patients Group		Control Group	
	Toxo-IgG		Toxo-IgG	
	Positive	Negative	Positive	Negative
5 - 14	0	4	0	4
15 - 24	8	12	5	15
25 - 34	9	15	7	13
35 - 44	9	10	8	12
45 - 54	6	7	7	9

[a] $P \leq 0.05$ is significant.
[b] P value (age) = Logestic Regression = 0.678.

these patients in Iran (20). In another study by Sundar, he showed a significant positive correlation between kala azar and toxoplasmosis in India (21). In a research, conducted in Norway by Kapperud, he found that *T. gondii* is a risk factor for miscarriage in pregnant women (22). Romano showed that people with *Chlamydia trachomatis* infection get better after infection with *T. gondii* due to consumption of cholesterol by Toxoplasma, which is essential for *Chlamydia trachomatis* (23). In some, studies the seroepidemiology of *Toxoplasma* infection with some other parasitic infections was evaluated. In a study by Cardia et al. in Brazil on the prevalence of IgG antibodies against *Toxoplasma* in cats with seropositivity to CL, a relationship was found with the cats' age and race (24). In another study conducted in Santiago, on mice with and without *T. gondii* infection, it was demonstrated that CL infection was lower in non-infected mice and they had a longer life (25). Although our research results didn't show a relationship between the two parasitic protozoan infections, we don't refuse the presence of a probable relationship between these two infections. This may indicate the need for more samples, and consideration of some side agents as the *Leishmania* and *Toxoplasma* species and the time post infection in CL patients. Therefore, we recommend a new study with more individual patients. In addition, we should control other agents, which may interfere with the results.

Our findings showed that there isn't any significant relationship between the prevalence of toxoplasmosis and cutaneous Leishmaniasis. Furthermore, the prevalence of *Toxoplasma* antibodies in patients with cutaneous Leishmaniasis by age and gender was almost equal and a relationship was not found.

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Footnote

Authors' Contribution: Abbasali Eskandarian, Hossein Hejazi and Vahid Raissi: experimental design of the study; Vahid Raissi, Hossein Yousefi and Somayeh Jahani: execution techniques and parasitological examination; Vahid Raissi and Abbasali Eskandarian: statistical analysis and collection of samples; all authors reviewed and contributed to the writing of this manuscript.

References

1. Carron AT, Lynn J, Keaney P. End-of-life care in medical textbooks. *Ann Intern Med.* 1999;**130**(1):82–6. [PubMed: 9890873].
2. Müller R, Baker JR. Medical parasitology. Lippincott Williams & Wilkins; 1990.
3. Koblansky AA, Jankovic D, Oh H, Hieny S, Sungnak W, Mathur R. Recognition of profilin by Toll-like receptor 12 is critical for host resistance to Toxoplasma gondii. *Immunity.* 2013;**38**(1):119–30.
4. Mohaghegh MA, Yazdani H, Hadipour M, Namdar F, Azami M, Kalani H, et al. Seroprevalence of Toxoplasma Gondii Infection among Patients Admitted to Al-Zahra Hospital, Isfahan, Iran. *J Ayub Med Coll Abbottabad.* 2015;**27**(4):767–70. [PubMed: 27004318].
5. Zhou P, Chen Z, Li HL, Zheng H, He S, Lin RQ, et al. Toxoplasma gondii infection in humans in China. *Parasit Vectors.* 2011;**4**:165. doi: 10.1186/1756-3305-4-165. [PubMed: 21864327].
6. Yolken RH, Bachmann S, Ruslanova I, Lillehoj E, Ford G, Torrey EF, et al. Antibodies to Toxoplasma gondii in individuals with first-episode schizophrenia. *Clin Infect Dis.* 2001;**32**(5):842–4. doi: 10.1086/319221. [PubMed: 11229859].
7. VanWormer E, Miller MA, Conrad PA, Grigg ME, Rejmanek D, Carpenter TE, et al. Using molecular epidemiology to track Toxoplasma gondii from terrestrial carnivores to marine hosts: implications for public health and conservation. *PLoS Negl Trop Dis.* 2014;**8**(5):ee2852. doi: 10.1371/journal.pntd.0002852. [PubMed: 24874796].

8. Cuccato LP, Lemos LL, de Souza MB, Ferreira Junior A, Fonseca BB, Mastrantonio EC. Biology and epidemiology of Toxoplasma gondii review paper. *PUBVET.* 2013;**7**(12).
9. Son ES, Nam HW. Detection and characterization of excretory/secretory proteins from Toxoplasma gondii by monoclonal antibodies. *Korean J Parasitol.* 2001;**39**(1):49–56. [PubMed: 11301590].
10. Gebremedhin EZ, Abdurahaman M, Tessema TS, Tilahun G, Cox E, Goddeeris B, et al. Isolation and genotyping of viable Toxoplasma gondii from sheep and goats in Ethiopia destined for human consumption. *Parasit Vectors.* 2014;**7**:425. doi: 10.1186/1756-3305-7-425. [PubMed: 25190185].
11. Eskandarian AA, Jafarnezghad GA, Akbari M. Seroprevalence of toxoplasma-specific antibodies in patients suspected to have active toxoplasmosis: A cross-sectional survey. *Adv Biomed Res.* 2014;**3**:236. doi: 10.4103/2277-9175.145738. [PubMed: 25538922].
12. Murray HW, Berman JD, Davies CR, Saravia NG. Advances in leishmaniasis. *Lancet.* 2005;**366**(9496):1561–77. doi: 10.1016/S0140-6736(05)67629-5. [PubMed: 16257344].
13. Hajjaran H, Mohebali M, Razavi MR, Rezaei S, Kazemi B, Edrissian G, et al. Identification of Leishmania species isolated from human cutaneous leishmaniasis, using random amplified polymorphic DNA (RAPD-PCR). *Iran J Public Health.* 2004;**33**(4):8–15.
14. Eslami G, Salehi R, Khosravi S, Doudi M. Genetic analysis of clinical isolates of Leishmania major from Isfahan, Iran. *J Vector Borne Dis.* 2012;**49**(3):168–74. [PubMed: 23135012].
15. Daryani A, Sarvi S, Aarabi M, Mizani A, Ahmadpour E, Shokri A, et al. Seroprevalence of Toxoplasma gondii in the Iranian general population: a systematic review and meta-analysis. *Acta Trop.* 2014;**137**:185–94. doi: 10.1016/j.actatropica.2014.05.015. [PubMed: 24887263].
16. Momeni AZ, Aminjavaheri M. Clinical picture of cutaneous leishmaniasis in Isfahan, Iran. *Int J Dermatol.* 1994;**33**(4):260–5. [PubMed: 8021082].
17. Jones JL, Kruszon-Moran D, Won K, Wilson M, Schantz PM. Toxoplasma gondii and Toxocara spp. co-infection. *Am J Trop Med Hyg.* 2008;**78**(1):35–9. [PubMed: 18187782].
18. Pearce BD, Kruszon-Moran D, Jones JL. The relationship between Toxoplasma gondii infection and mood disorders in the third National Health and Nutrition Survey. *Biol Psychiatry.* 2012;**72**(4):290–5. doi: 10.1016/j.biopsych.2012.01.003. [PubMed: 22325983].
19. Shirbazou S, Delpisheh A, Mokhetari R, Tavakoli G. Serologic Detection of Anti Toxoplasma gondii Infection in Diabetic Patients. *Iran Red Crescent Med J.* 2013;**15**(8):701–3. doi: 10.5812/ircmj.5303. [PubMed: 24578838].
20. Mohraz M, Mehrkhani F, Jam S, SeyedAlinaghi S, Sabzvari D, Fattahi F, et al. Seroprevalence of toxoplasmosis in HIV(+)/AIDS patients in Iran. *Acta Med Iran.* 2011;**49**(4):213–8. [PubMed: 21713730].
21. Sundar S. Diagnosis of kala-azar–an important stride. *J Assoc Physicians India.* 2003;**51**:753–5. [PubMed: 14651132].
22. Kapperud G, Jenum PA, Stray-Pedersen B, Melby KK, Eskild A, Eng J. Risk factors for Toxoplasma gondii infection in pregnancy. Results of a prospective case-control study in Norway. *Am J Epidemiol.* 1996;**144**(4):405–12. [PubMed: 8712198].
23. Romano JD, de Beaumont C, Carrasco JA, Ehrenman K, Bavoil PM, Coppens I. A novel co-infection model with Toxoplasma and Chlamydia trachomatis highlights the importance of host cell manipulation for nutrient scavenging. *Cell Microbiol.* 2013;**15**(4):619–46. doi: 10.1111/cmi.12060. [PubMed: 23107293].
24. Cardia DF, Camossi LG, Neto Lda S, Langoni H, Bresciani KD. Prevalence of Toxoplasma gondii and Leishmania spp. infection in cats from Brazil. *Vet Parasitol.* 2013;**197**(3-4):634–7. doi: 10.1016/j.vetpar.2013.07.017. [PubMed: 23932640].
25. Santiago HC, Oliveira MA, Bambirra EA, Faria AM, Afonso LC, Vieira LQ, et al. Coinfection with Toxoplasma gondii inhibits antigen-specific Th2 immune responses, tissue inflammation, and parasitism in BALB/c mice infected with Leishmania major. *Infect Immun.* 1999;**67**(9):4939–44. [PubMed: 10456955].