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Original paper

Toxoplasma gondii infection in patients with rheumatoid arthritis and systemic lupus erythematous diseases: serological and molecular evidence

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ABSTRACT. This study aimed to determine *Toxoplasma gondii* infection in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) patients in Mazandaran Province, north of Iran. During April 2019 to February 2020, 305 patients with RA (N=272) and SLE (N=33) diseases are included in this cross-sectional study. The baseline data of patients were collected into a questionnaire. Also, blood sample was taken from each patient in tubes containing k2-EDTA as anticoagulant for serological and molecular analyses. The presence of specific IgG antibody against *T. gondii* in plasma was explored using ELISA method. DNA was extracted and conventional-PCR method was done using RE primers on buffy coat samples. Anti-*Toxoplasma* IgG were detected among 105/272 RA (38.6%) and 11/33 (33%) SLE patients (P=0.55). IgG seropositivity was more common in females (95.70%) than in males (4.30%) and in 51–60 years old patients (37.10%) in rural populations (62.07%). Also, seropositivity was higher in patients who kept cats (93.95%). Keeping cats and patient's age were two studied risk factors which had significant relations with *T. gondii* seropositivity in patients (P<0.05). Overall, *T. gondii* DNA was found in 60 of 305 (19.7%) of enrolled patients, whether serology positive or negative (P<0.0001). Given the RA and SLE patients can be considered as a risk group for toxoplasmosis, in addition to the serological test, PCR based techniques is recommended for early and accurate detection of recent *T. gondii* infection.

Keywords: *Toxoplasma gondii*, rheumatoid arthritis, systemic lupus erythematosus

Introduction

Toxoplasma gondii (*T. gondii*) is an incredibly successful parasite responsible for a neglected parasitic disease, toxoplasmosis, which differs extremely in its clinical form, ranging from asymptomatic cases to fatal consequences in population [1,2]. It is estimated that as at least one-third of the world's humans are infected with *Toxoplasma* parasite in both developed and developing countries [3].

Toxoplasmosis is an inflammatory disease that Th1-type immune response with the contribution of pro-inflammatory cytokines and a humoral response which results in production of specific anti-*Toxoplasma* antibodies detected during infection [4]. The production of these pro-inflammatory cytokines, restrict reproduction of *T. gondii* tachyzoites, but the protozoa forms a chronic stage by organization of cysts in toxoplasma infected individuals. These tissue cysts stay frequently dormant in the body and may become reactivated

later in life [5]. With the knowledge advance of *Toxoplasma* parasite and its relationship with the immune system, it has already been noted that toxoplasmosis may contribute to the development of many autoimmune disorders through immunological cross-reactivity between the parasite and components of host tissues [6].

Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), the chronic autoimmune autoinflammatory disorders, characterized with the production of auto-antibodies and systemic effects that the genetic and environmental factors often affect the emergence of diseases [7–10]. There is also reports from previous studies that showed the relationship between *Toxoplasma* infection and autoimmune diseases such as RA and SLE [8,11,12].

Due to lack of information about the status of *T. gondii* infection in patients with autoimmune diseases in the region and also no vaccine is presented to barricade toxoplasmosis in individuals; the present study was attempted, for the first time, to survey *Toxoplasma* infection among new patients suffering from autoimmune diseases (RA and SLE) who referred to the subspecialty clinics (Mostafavian and Tooba) in Mazandaran Province, Sari, north of Iran.

Materials and Methods

Ethical approval

This survey was acquiesced by the joint Ethical Committees of Mazandaran University of Medical Sciences (Ethic No. IR.MAZUMS.REC.1398. 5627).

Study design and study population

This cross-sectional study was included the patients with defined clinical forms of RA and SLE diseases (as new case), referred to subspecialty clinics (Mostafavian and Tooba) in Mazandaran Province, Sari, northern Iran, between April 2019 and February 2020. Baseline data which included demographics, age, sex, place of residence, marital status, level of education, underlying disease, keeping the pets and type of disease (RA/SLE) were collected into a questionnaire. Then, blood samples were collected from 272 and 33 patients with prior diagnosis of RA and SLE, respectively, in tubes containing k2-EDTA as anticoagulant and centrifuged at 3,000 rpm for 3 min. Afterward, the plasma and buffy coat were accumulated at -20°C for further examination.

Serological assay

The presence of specific IgG antibody against *T. gondii* was explored using a commercially available enzyme-linked immune sorbent assay (ELISA) kit (PishtazTeb, Iran), based on the manufacturer's protocol. The status of toxoplasmosis was determined according to the formula on the kit. The results > 1.1 was considered positive.

Molecular assay

T. gondii DNA were extracted from the 305 specimens according to the phenol chloroform isoamidalcol method [13]. Then, Conventional-PCR (PCR) was done by forward primer F 5′-CGCTCA GGGAGGAAGACGAAAGTTG-3′ and reverse primer R 5′-CGCTGCAGACACAGTGCATCTG G ATT-3′, amplifying a 529-bp fragment of gene RE with master mix (12.5 µl; Fermentas) mixed with 4 µl of the extracted DNA to accede an ultimate content of 25 ml comprising 7.3 µl distilled water and 0.6 µl of each primer at a concentration of 1 pmol/µl. Then, 32 cycles were performed in a thermocycler (Corbett Research, Sydney, Australia) at 94°C, for 3 min by 1 cycle (initiation denaturation), 94°C for 30 s (denaturation), 55°C for 30 s (annealing), 72°C for 20 s (extension), 30 cycles and 72°C for 7 min 1 cycle (final extension). Subsequently, PCR product was analyzed by electrophoresis on 1% (w/v) agarose gel in Tris-borate-EDTA at 85 V for 25 min and observed using UV transillumination after staining with Safe View™ DNA Stains (Applied Biological Materials, Inc.).

Data analysis

Statistical analysis was analyzed using SPSS software (version 23.0). Frequency of data was showed in percentages (%). The extended Fisher test and Chi square test was used to exam statistically significant differences for parametric data. A *P*-value of less than 0.05 was considered statistically significant.

Results

Out of 305 RA and SLE patients, consisting of 23 males and 282 females, IgG seropositivity were detected among 105/272 RA (38.6%) and 11/33 (3%) SLE patients (P=0.55), more common in females (95.70%) than in males (4.30%) and in 51–60 years old patients (37.10%) in rural populations (62.07%). Also, 20% of seropositive

Table 1. Demographic characteristics and anti-*Toxoplasma* specific IgG antibody results of RA and SLE patients in Mazandaran, northern Iran

Characteristics	Overall	*T. gondii* IgG		*P*-value
		Negative	Positive	
Gender				
Male	23 (7.50)	18 (9.50)	5 (4.30)	0.09
Female	282 (92.50)	171 (90.50)	111 (95.70)	
Age				
<20	2 (0. 65)	0 (0.00)	2 (1.70)	0.03*
20–30	15 (4.90)	11 (5.80)	4 (3.45)	
31–40	42 (13.80)	32 (16.95)	10 (8.60)	
41–50	59 (19.35)	37 (19.58)	22 (18.95)	
51–60	98 (32.13)	55 (29.10)	43 (37.10)	
61–70	69 (22.62)	46 (24.35)	23 (19.85)	
>70	20 (6.55)	8 (4.23)	12 (10.35)	
Residential place				
Urban	124 (40.65)	80 (42.30)	44 (37.93)	0.44
Rural	181 (59.35)	109 (57.60)	72 (62.07)	
Marital status				
Married	290 (95.00)	181 (95.75)	109 (93.95)	0.48
Single	15 (5.00)	8 (4.25)	7 (6.05)	
Education				
Illiterate	59 (19.35)	39 (20.63)	20 (17.25)	0.06
Elementary	89 (29.20)	45 (23.80)	44 (37.93)	
Secondary (High school)	121 (39.65)	79 (41.81)	42 (36.20)	
Callegiate level	36 (11.80)	26 (13.76)	10 (8.62)	
Past medical history				
Diabetes mellitus	46 (15.10)	28 (14.80)	18 (15.51)	0.11
Kidney failure	15 (4.90)	13 (6.88)	2 (1.72)	
Cardiovascular	22 (7.20)	10 (5.30)	12 (10.36)	
Cirrhosis of the liver	1 (0.32)	0 (0.00)	1 (0.86)	
Hypothyroidism	19 (6.22)	14 (7.40)	5 (4.32)	
Asthma	4 (1.31)	2 (1.06)	2 (1.72)	
None	198 (64.95)	122 (64.56)	76 (65.51)	
Keeping cats				
Yes	134 (94.80)	73 (38.60)	109 (93.95)	0.01*
No	171 (5.20)	116 (61.40)	7 (6.05)	
Type of disease				
RA	272 (89.20)	167 (61.4)	105 (38.6)	0.55
SLE	33 (10.80)	22 (66.7)	11 (33)	

subjects had diabetes mellitus. Furthermore, seropositivity was higher in patients who kept cats (93.95%).

A significant difference was observed between the IgG seropositivity and age (P=0.03), and IgG test result and keep pets (P=0.01). Table 1 displays the comparison of demographics data in IgG seropositive and seronegative groups in 272 RA and 33 SLE patients. Surprisingly, PCR outcomes were positive in 23 of 116 (19.8%) and in 37 of 189 (19.6%) IgG seropositive and seronegative groups, respectively (P<0.0001). Overall, 19.7% (60/305) of enrolled patients, whether serology positive or negative, were PCR positive.

Discussion

Based on the previous studies, a high prevalence of chronic toxoplasmosis (59%) was reported in northern Iran [14]. In this project have focused on the serological and molecular prevalence of toxoplasmosis in autoimmune diseases (RA and SLE), in this regain for the first time. In the present study, consistent with the findings of other studies, autoimmune diseases were more common in females (95.70%) than in males (4.30%); although a significant difference was not observed according gender. In Ismail et al. [15] study 83% of autoimmune patients were female. Also, Nikpour et al. [16], in a systematic review reported that SLE is particularly prevalent in Australian women in childbearing age.

In this study of 305 subjects, anti-*Toxoplasma* IgG was positive in 38.6% and 33% RE and SLE patients respectively, although a significant difference was not observed between two groups.

In a study recently evaluated by Ismail et al. [15], of 90 autoimmune disease Saudi patients anti-*Toxoplasma* IgG was positive in 22.2% cases and a highly significant difference was observed between case and control groups (P<0.001).

Serological detection is the primary method for the diagnosis of *T. gondii*-specific antibody, which indicate earlier exposure. Therefore, molecular methods are recognized as critical diagnostic too with high sensitivity and specificity, especially for acute toxoplasmosis diagnosis [17]. In our investigation, *T. gondii* DNA was detected in 19.6% of seronegative patients by molecular diagnosis indicated the acute stage of the infection. The presence of *T. gondii* most possibly represent a parasitaemia in the peripheral blood or recent infection [18]. Therefore, these patients needed to be monitored in this regard and should be subjected to treatment. Also, both anti-*Toxoplasma* IgG and PCR negative cases (80.4%) have no signal of prior exposure to *T. gondii* parasite and consequently have a high risk of infection. Therefore, they will need serial testing and follow-up. Moreover, 80.2% of patients showed no evidence of *T. gondii* infection by PCR, though IgG antibodies were detected. This situation most possibly represents a long standing immunity against parasite [18].

Given that toxoplasmosis can be as a risk in patients with RA and SLE, reactivation of the toxoplasma cysts during treatment of these patients with immunosuppressive drugs cause severe consequences, toxoplasmic encephalitis and even activation of immune modulators in these cases [19–21]. Also, consumption of immunosuppressive drugs by these patients blocking the secretion of TNF-α [4,5], a Th1 response cytokine that plays a significant role in the resistance to acute and chronic *Toxoplasma* infection [22,23]. On the other hand, recent treatments with anti-TNF-α, leads to brain toxoplasmosis in RA patients [21].

Moreover, the activation of some toll-like receptors and inflammatory response in *T. gondii* infection enhances manufacture of autoimmune antibodies [24]. Furthermore, *T. gondii* parasite increases the expression of interleukin 17 in infected individuals. This cytokine is involved in the pathogenesis of many autoimmune illnesses and thus a significant relationship between toxoplasmosis and autoimmune diseases can be explained [21,25]. All these factors, can contribute to the progression of chronic diseases quickly and cause an acute toxoplasmosis that threaten the patient's life [26].

Cats are the key animal species that can excrete environmentally resistant oocysts especially in a warm and humid areas and thus play a pivotal role in the epidemiology of *T. gondii* infection [27–30]. In humans, accidental ingestion of sporulated oocysts shed in soil and consumption of raw or undercooked meat containing *T. gondii* tissue cysts are the main routes of transmission [31,32]. Therefore, in patients with RA and SLE diseases, prevention and screening programs and so treatment of toxoplasmosis should be given more consideration.

In conclusion, given the possible role of toxoplasmosis in incidence and aggravating the symptoms of the RA and SLE diseases, in addition to the serologic tests, PCR based technique is

recommended early and true detection of recent *T. gondii* infection in these patients. Also, further studies will be necessary to clarify the new mechanism pathogenesis of *T. gondii* in the development of autoimmune diseases.

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References

[1] Dubey J. 2009. History of the discovery of the life cycle of *Toxoplasma gondii. International Journal for Parasitology* 39: 877-882. doi:10.1016/j.ijpara.2009.01.005

[2] Hotez P.J. 2014. Neglected parasitic infections and poverty in the United States. *PLoS Neglected Tropical Diseases* 8: e3012. https://doi.org/10.1371/journal.pntd.0003012

[3] Flegr J., Preiss M., Klose J., Havlíček J., Vitáková M., Kodym P. 2003. Decreased level of psychobiological factor novelty seeking and lower intelligence in men latently infected with the protozoan parasite *Toxoplasma gondii* dopamine, a missing link between schizophrenia and toxoplasmosis? *Biological Psychology* 63: 253-268. https://doi.org/10.1016/s0301-0511(03)00075-9

[4] Filisetti D., Candolfi E. 2004. Immune response to *Toxoplasma gondii*. *Annali dell' Istituto Superiore di Sanita* 40: 71-80.

[5] Suzuki Y., Wang X., Jortner B.S., Payne L., Ni Y., Michie S.A., Xu B., Kudo T., Perkins S. 2010. Removal of *Toxoplasma gondii* cysts from the brain by perforin-mediated activity of $CD8^+$ T cells. *The American Journal of Pathology* 176: 1607-1613. https://doi.org/10.2353/ajpath.2010.090825

[6] Dupont C.D., Christian D.A., Hunter C.A. 2012. Immune response and immunopathology during toxoplasmosis. *Seminars in Immunopathology* 34: 793-813. doi:10.1007/s00281-012-0339-3

[7] Cribbs A., Feldmann M., Oppermann U. 2015. Towards an understanding of the role of DNA methylation in rheumatoid arthritis: therapeutic and diagnostic implications. *Therapeutic Advances in Musculoskeletal Disease* 7: 206-219. https://doi.org/10.1177/1759720X15598307

[8] Fischer A.L.N., ShapiraY., Katz B.S., Porat G., Eduard C., Ricard S., Ljudmila P., Jose A.G., Sanmartí R., Shoenfeld Y. 2013. *Toxoplasma gondii*: bystander or cofactor in rheumatoid arthritis. *Immunologic Research* 56: 287-292. https://doi.org/10.1007/s12026-013-8402-2

[9] McInnes I., Schett G. 2011. The pathogenesis of rheumatoid arthritis. *New England Journal of Medicine* 365: 2205-2219. https://doi.org/10.1056/nejmra1004965

[10] Periasamy P., Byrd V.L. 2019. Generative adversarial networks for lupus diagnostics, In: *Proceedings of the Practice and Experience in Advanced Research Computing on Rise of the Machines* (learning): 1-8. https://doi.org/10.1145/3332186.3338102

[11] El-Sayed N.M., Kishik S.M., Fawzy R.M. 2016. The current status of *Toxoplasma gondii* infection among Egyptian rheumatoid arthritis patients. *Asian Pacific Journal of Tropical Disease* 6: 797-801. https://doi.org/10.1016/S2222-1808(16)61133-7

[12] El-Henawy A.A., Hafez E.A.R., Nabih N.S., Naglaa M., Mervat M. 2017. Anti-*Toxoplasma* antibodies in Egyptian rheumatoid arthritis patients. *Rheumatology International* 37: 785-790. https://doi.org/10.1007/s00296-017-3703-8

[13] Moazeni J.F., Moazeni J.G., Nozari N., Kavari H., Hashemzadeh F.H. 2013. A serological and molecular study on *Toxoplasma gondii* infection in sheep and goat in Tabriz. *Archives of Razi Institute* 68: 29-35

[14] Daryani A., Sarvi S., Aarabi M., Mizani A., Ahmadpour E., Shokri A., Rahimi M.T., Sharif M. 2014. Seroprevalence of *Toxoplasma gondii* in the Iranian general population: a systematic review and meta-analysis. *Acta Tropica* 137: 185-194. https://doi.org/10.1016/j.actatropica.2014.05.015

[15]Ismail K., Zaini R., Gharib A.F., Hagag H.M., Hassan A.F., Alzahrani S., Aied A., Alhomaidi F., Mohammed N., Abdulaziz S., Aljumaihe S. 2019. Seroprevalence of *Toxoplasma gondii* in Saudi autoimmune disease patients. *Annals of Immunology & Immunotherapy* 1: 1-4. https://doi.org/10.23880/aii-16000102

[16] Nikpour M., Bridge J., Richter S. 2014. A systematic review of prevalence, disease characteristics and management of systemic lupus erythematosus in Australia: identifying areas of unmet need. *Internal Medicine Journal* 44: 1170-1179. https://doi.org/10.1111/imj.12568

[17] Remington J.S., Thulliez P., Montoya J.G. 2004. Recent developments for diagnosis of toxoplasmosis. *Journal of Clinical Microbiology* 42: 941-945. https://dx.doi.org/10.1128/JCM.42.3.941-945.2004

[18] Garweg J., Boehnke M., Koerner F. 1996. Restricted applicability of the polymerase chain reaction for the diagnosis of ocular toxoplasmosis. *German Journal of Ophthalmology* 5: 104-108.

[19] Furuya H., Ikeda K., Iida K., Suzuki K., Furuta S., Tamachi T., Suzuki K., Miura G., Hiraguri M., Hase R. 2019. Disseminated toxoplasmosis with atypical symptoms which developed with exacerbation of systemic lupus erythematosus. *Lupus* 28: 133-136. https://doi.org/10.1177/0961203318815583

[20] Lewis J.M., Clifford S., Nsutebu E. 2015. Toxoplasmosis in immunosuppressed patients. *Reumathology* (*Oxford University*) 54: 1939-1940. https://doi.org/10.1093/rheumatology/kev115

[21] Hosseininejad Z., Sharif M., Sarvi S., Amouei A., Hosseini S.A., Chegeni T.N., Anvari D., Saberi R., Gohardehi S., Mizani A., Sadeghi M. 2018. Toxoplasmosis seroprevalence in rheumatoid arthritis

patients: a systematic review and meta-analysis. *PLoS Neglected Tropical Diseases* 12: e0006545. https://doi.org/10.1371/journal.pntd.0006545

[22] Deckert-Schlüter M., Bluethmann H., Rang A., Hof H., Schlüter D. 1998. Crucial role of TNF receptor type 1 (p55), but not of TNF receptor type 2 (p75), in murine toxoplasmosis. *The Journal of Immunology* 160: 3427-3436.

[23] Yap G.S., Scharton-Kersten T., Charest H., Sher A. 1998. Decreased resistance of TNF receptor p55-and p75-deficient mice to chronic toxoplasmosis despite normal activation of inducible nitric oxide synthase *in vivo*. *The Journal of Immunology* 160: 1340-1345.

[24] Bach J.F. 2002. The effect of infections on susceptibility to autoimmune and allergic diseases. *New England Journal of Medicine* 347: 911-920. doi:10.1056/nejmra020100

[25] Gaffen S.L. 2009. The role of interleukin-17 in the pathogenesis of rheumatoid arthritis. *Current Rheumatology Reports* 11: 365-70. https://doi.org/10.1007/s11926-009-0052-y

[26] Luft B.J., Remington J.S. 1992. Toxoplasmic encephalitis in AIDS. *Clinical Infectious Diseases* 15: 211-222. https://doi.org/10.1093/clinids/15.2.211

[27] Sharif M., Daryani A., Nasrolahei M., Ziapour S.P. 2009. Prevalence of *Toxoplasma gondii* antibodies in stray cats in Sari, northern Iran. *Tropical Animal Health and Production* 41: 183-187. https://doi.org/10.1007/s11250-008-9173-y

[28] Egorov A.I., Converse R., Griffin S.M., Styles J., Klein E., Sams E. Hudgens E., Wade T.J. 2018. Environmental risk factors for *Toxoplasma gondii* infections and the impact of latent infections on allostatic load in residents of Central North Carolina. *BMC Infectious Diseases* 18: 1-11.

[29] Elmore S.A., Jones J.L., Conrad P.A., Patton S., Lindsay D.S., Dubey J.P. 2010. *Toxoplasma gondii*: epidemiology, feline clinical aspects, and prevention. *Trends in Parasitology* 26: 190-196. https://doi.org/10.1016/j.pt.2010.01.009

[30]Meerburg B.G., Kijlstra A. 2009. Changing climate-changing pathogens: *Toxoplasma gondii* in North-Western Europe. *Parasitology Research* 105: 17-24. https://doi.org/10.1007/s00436-009-1447-4

[31] Montazeri M., Galeh T.M., Moosazadeh M., Sarvi S., Dodangeh S., Javidnia J., Sharif M., Daryani A. 2020. The global serological prevalence of *Toxoplasma gondii* in felids during the last five decades (1967–2017): a systematic review and meta-analysis. *Parasites & Vectors* 13: 1-10. https://doi.org/10.1186/s13071-020-3954-1

[32] Gebremedhin E.Z., Abdurahaman M., Tessema T.S., Tilahun G., Cox E., Goddeeris B., Dorny P., De Craeye S., Dardé M.L., Ajzenberg D. 2014. Isolation and genotyping of viable *Toxoplasma gondii* from sheep and goats in Ethiopia destined for human consumption. *Parasites & Vectors* 7: 1-8. doi.org/10.1186/1756-3305-7-425

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