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Original Article

Toxoplasmosis Frequency Rate in Rheumatoid Arthritis Patients in Northeastern Iran

Mehdi Zarean [1], Pietro Mastroeni [2], *Elham Moghaddas [1], *Bibi Razieh Hosseini Farash [1], Amene Raouf-Rahmati [1], Jamshid Jamali [3], Hossein Azadeh[4], Vahideh Kam [1]

1. *Department of Parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
2. *Department of Veterinary Medicine, Cambridge University, Cambridge, United Kingdom*
3. *Department of Biostatistics and Epidemiology, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran*
4. *Department of Internal Medicine, Rheumatology Division, Orthopaedic Research Center, Mazandaran University of Medical Sciences, Sari, Iran*

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***Correspondence**
Email:
Moghaddase@mums.ac.ir;
hoseinifr@mums.ac.ir

Abstract

Background: Toxoplasmosis is a zoonotic disease caused by the parasite *Toxoplasma gondii,* a cosmopolitan intracellular parasite. It can be a risk factor for autoimmune diseases, including rheumatoid arthritis (RA). This study was designed to investigate the possible association between serological history of *T. gondii* infection and defined clinical manifestation of RA in Northeast of Iran.

Methods: Overall, serum samples were collected from 50 RA patients and 40 healthy controls, from Qaem Hospital in Mashhad City, northeastern Iran in 2018. Seroprevalence of *T. gondii* infection was determined by ELISA.

Results: The prevalence of anti -*T. gondii* IgG in RA patients 48% (24.50) was significantly higher than the control group 10% (4.40) (P <0.001). Erythrocyte sedimentation rate (ESR), anti-cyclic citrullinated peptide (anti-CCP) and (rheumatoid factor) RF levels between the RA and control groups (P <0.01). Control group were matched with patients for age, gender and living area.

Conclusion: Given that a high correlation has been demonstrated between positivity rate of anti-*T. gondii* IgG and RA in Northeastern Iran, further studies will be necessary to clarify the pathogenesis of *T. gondii* among these patients.

Introduction

Toxoplasmosis is an intracellular parasitic disease caused by *Toxoplasma gondii.* About one third of the global population are infected by this parasite. Tissue cysts of the parasite and sporulated oocysts can infect humans through consumption of raw or

semi-cooked meat or contaminated water and food. Vertical transmission during pregnancy is another route of *Toxoplasma* transmission from placenta to the fetus (1). *T. gondii* infection is asymptomatic in the early stage of infection in individuals who are immunocompetent. However, sever clinical manifestations and even death have been observed in immunocompromised patients or during pregnancy (2).

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that a dysregulates immune response attacks the tissues. The inflammation targets the joints and synovial tissues (arthritis) and can extend to parts of the body like inflammation around the lungs and the heart (3). Changes in innate and adaptive immune responses in patients with RA could be associated with a high risk of toxoplasmosis as an opportunistic infection (4). Moreover, the patients who suffer from RA are treated with TNF-α inhibitors to provide effective immunosuppression (5). On the other hand, experimental evidence showed the role of infectious diseases in development of autoimmune disorders via epitope spreading molecular mimicry (6).

Production of pro-inflammatory cytokines in toxoplasmosis, such as tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ), limit the growth of the parasite but also mediate some of the clinical signs of acute toxoplasmosis (7, 8). Some cytokine production profiles in autoimmune diseases raises the possibility of some bacterial and parasitic infection, including toxoplasmosis (9). In addition, RA is exacerbated by high level of Th1 without sufficient Th2 generation (10) and toxoplasmosis leads to overproduction of Th1 cytokines (11). So, simultaneous occurrence of these diseases can potentially exacerbate RA (12).

Because of the high- prevalence of toxoplasmosis and RA in Iran, we aimed to investigate the possible association between *T. gondii* infection, RA, and risk factors for infection with this parasite for the first time in patients with defined clinical manifestation of RA in Northeast of Iran.

Materials and Methods

Patients

In this case–control study, 90 participants on 50 RA patients and 40 healthy controls referred to Qaem Hospital in Mashhad City, from March to December 2018 were enrolled. RA patients diagnosed according to the American College of Rheumatology (ACR) criteria (Table 1). Control group was the one of the family members who came to the hospital with the patient. They were free of any chronic pain and other chronic inflammatory diseases and without using anti rheumatic drugs (DMARDs) or steroids. Control group were matched with patients for age, gender and living area.

Sample size was calculated considering a prevalence of 58% (10), relative precision of 4 (d=0.04), type I error or alpha 0.05, power 80, and potential attrition rate 10%. Sera were kept frozen at -20 °C until use. Rheumatoid factors (RF), anti-cyclic citrullinated peptide (CCP) antibody and erythrocyte sedimentation rate (ESR) that used in the diagnosis of rheumatoid arthritis were evaluated in these patients (13, 14).

Ethical considerations

Ethics approval was obtained through the Ethics Committee at Mashhad University of Medical Sciences (Ethical code: IR.MUMS.MEDICAL.REC.1397.755). Informed written consent was obtained from all patients and healthy individuals.

IgG-ELISA

Anti-*T. gondii* IgG antibodies were measured using a commercial ELISA kit (Pishtaz Teb Zaman, Tehran, Iran) according to the instructions of the manufacture. Optical density was recorded at 450nm and 360nm with an automated ELISA reader (BIOTEC, LX800,

USA). Based on kit protocol, results higher than 1.1 should be considered as positive and results less than 0.9 are considered as negative. The values between 0.9 and 1.1 are considered as equivocal and the test should be repeated after a while with fresh specimen.

The specificity and sensitivity of the kits were 99 and 100 %, respectively. To assess the reliability of the kits, inter- and intra-assay were evaluated and produced scores of CV < 14% and CV < 3%, respectively.

Statistical analysis

Statistical analysis was conducted using the SPSS version 25 (IBM Corp., Armonk, NY, USA). Normally distributed quantitative variables were demonstrated as mean ± standard deviation. For statistical analysis, the Kolmogorov–Smirnov test to evaluate normality condition, chi-square test and Fisher's exact test to access relation between quantities' variable, and *t* test to access deference between two groups were used. For all statistical analyses, a *P*-value less than 0.05 was considered significant.

Results

We observed significant differences in ESR, anti-CCP and RF levels between the RA and control groups (*P* <0.01; Table 1).

Table 1: ESR, anti-CCP and RF levels in patients with RA and healthy controls

Variables	*RA*	*Control*	*P-value*
ESR (mm/h)	27.3±3	9.8±4	<0.001
Anti-CCP (RU/mL)	334±5	28.2±2	<0.001
RF (IU/mL)	44.7±3	13±3	<0.001

The overall seroprevalence of IgG antibodies against *T. gondii* infection was 48% (24/50) and 10% (4/40) in RA patients and control group, respectively (*P* <0.001). The mean of age in case and control groups were 48.02± 13.69 and 47.11± 9.83 years, respectively. There were no differences between the age groups (*P*=0.719).

Other descriptive data on demographic status are shown in Table 2-3. No significant relationship was seen between toxoplasmosis and other tested variables.

Table 2: Distributions and descriptive statistics for demographic status in patients with RA and healthy controls

Variables		*RA(%)*	*Control(%)*	*Total(%)*	*P-value*
Sex	Male	8 (16.0)	2 (5.0)	10 (11.1)	0.175
	Female	42 (84.0)	38 (95.0)	80 (88.9)	
Resident	urban	26 (55.3)	28 (70.0)	54 (62.1)	0.160
	rural	24 (44.7)	12 (30.0)	36 (37.9)	
lgG	Positive	24 (48.0)	4 (10.0)	28 (31.1)	<0.001
	Negative	26 (52.0)	36 (90.0)	62 (68.9)	

Table 3: Demographic characteristics, risk factors and serological results of RA patients

Variables		*Positive IgG*	*Negative IgG*	*Total*	*P-value*
Sex	Male	2 (8.3%)	6 (23.1%)	8 (16.0%)	0.250
	Female	22 (91.7%)	20 (76.9%)	42 (84.0%)	
Level of education	Illiterate	7 (30.4%)	2 (8.3%)	9 (19.1%)	0.098
	Primary to high school	14 (60.9%)	16 (66.7%)	30 (63.8%)	
	Academic	2 (8.7%)	6 (25.0%)	8 (17.0%)	
Resident	Urban	15 (65.2%)	11 (45.8%)	26 (55.3%)	0.181
	Rural	8 (34.8%)	13 (54.2%)	21 (44.7%)	
Raw meat	Yes	1 (4.3%)	4 (16.7%)	5 (10.6%)	0.348
	No	22 (95.7%)	20 (83.3%)	42 (89.4%)	
Vegetables	Yes	2 (8.7%)	5 (20.8%)	7 (14.9%)	0.416
	No	21 (91.3%)	19 (79.2%)	40 (85.1%)	
Cat	Yes	1 (4.3%)	5 (20.8%)	6 (12.8%)	0.188
	No	22 (95.7%)	19 (79.2%)	41 (87.2%)	
Job	Housewife	19 (82.6%)	14 (58.3%)	33 (70.2%)	0.069
	Employed	4 (17.4%)	10 (41.7%)	14 (29.8%)	
Pica	Yes	3 (13.0%)	2 (8.3%)	5 (10.6%)	0.666
	No	20 (87.0%)	22 (91.7%)	42 (89.4%)	
Age		50.63±13.54	45.42±13.62	48.02±13.69	0.191
Disease duration (yr)		5.95±6.72	5.43±5.28	5.68±5.95	0.772
ESR		23.16±16.85	25.29±16.79	24.22±16.54	0.741
Anti-CCP		214.65±240.77	635.15±1095.39	433.31±820.05	0.200

Discussion

Toxoplasmosis is a common parasitic infection that is increasingly being reported in patients with RA; however, the impact of this parasite have not been completely clear (2). In present study, the frequency rate of *T. gondii* infection among 50 patients with RA were assessed.

IgG seropositivity showed that case-patients with RA were more likely to have *T. gondii* infection (48% in comparison with 10% of healthy controls, $P < 0.001$). Other studies have reported the statistical relationship between *T. gondii* infection and RA in other geographical areas, such as China (18.8%), Europe (63.0%), Iraq (54.0%), Tunisia (58.4%), and Egypt (54.0% and 76.7%) (15-17). The higher IgG antibody level against *T. gondii* in RA patients compared to healthy controls reveals a correlation between chronic toxoplasmosis and RA.

Our findings showed a prevalence of 10% IgG seropositivity for *T. gondii* infection in the control group. However, the meta-analysis was done by Iranian researchers obtained a prevalence of 21% for toxoplasmosis in the control group in some studies (12). This difference could be due to the different geographic areas, various eating habits and different age and sex compared to our study. In Iran, the most prevalence of anti *T. gondii* IgG was evaluated among 76 of 93 patients with RA (81.72%) versus 37 of 93 healthy control group (39.80%). In addition, the seroprevalence of anti *T. gondii* IgM was significantly higher in patients in RA (36 of 93; 38.70%) compared to the healthy control group (2 of 93; 2.1%) (18). There was no significant difference between RA patients and control

group with regard to sex and residential location (urban or rural) to be consistent with another research in China (2). In spite of several previous studies reporting a significant association between contact with cats and *T. gondii* seropositivity, we could not find the role of contact with cats as a risk factor in patients with *T. gondii* infection (2, 19, 20).

The results of this study indicated that *T. gondii* may induce a pathologic process in individuals, which can eventually result in RA or RA patients be prone to toxoplasmosis. Based on some data, this parasite can act as ligand for toll-like receptors (TLRs) and consequently has the ability to elicit inflammatory response (21). Besides, the rise of interleukin 17 expression (IL-17) has been reported in patients with toxoplasmosis. It seems that this cytokine is important to contribute in pathogenesis of many autoimmune disorders, including RA. Therefore, an obvious association between toxoplasmosis and RA can be described (22).

Moreover, the role of toxoplasmosis has been explained in progression of autoimmune diseases (AID). Immunosuppressive drug therapy increases the risk of infection in patients with autoimmune diseases (12). The higher prevalence of *T. gondii* has been reported in patients with other autoimmune disorders such as lupus erythematosus, diabetes mellitus, and autoimmune thyroid diseases (19, 23, 24).

Among 52 patients with multiple sclerosis, 23 (44.2%) were positive for anti-*T.gondii* IgG antibodies, which had a significant difference compared to the healthy control group (24.4%) (P=0.042) (25). Another study on autoimmune disease, reported a positive correlation between *Toxoplasma* infection and Type I diabetes (26). In that study, out of 91 diabetic patients, 26 (28.6%) had an IgG antibody to *T. gondii,* while it was 7 (7.7%) for the control group (P=0.001) (26). According to these studies, *T. gondii* infection may be one of the several environmental risk factors for Type I diabetes and multiple sclerosis. In comparison between 1514 patients with 11 different AID conditions that were collected from many referral centers from 437 geographically in Europe and America with matched controls, IgG anti- *T. gondii* were positive in patients with AID versus of controls significantly (6).

Overall, a high correlation between positivity rate of anti-*T. gondii* IgG and RF has been demonstrated in Northeastern Iran. This result shows an increased susceptibility to opportunistic *T. gondii* infection in patients with RF.

The question remains whether toxoplasmosis leads to develop RF; whether immunosuppression during the infection reactive a latent *T. gondii* infection; or other unknown reasons provide infection susceptibility to novel toxoplasmosis. Since drugs taken in treatment of arthritis patients may increase the risk of this parasitic infection, it is proposed latent toxoplasmosis screening in patients with RF before starting TNF-α inhibitors therapy.

Conclusion

Toxoplasmosis could be considered as a potential risk for RA patients. Maybe *T. gondii* be effective on induction or exacerbation of RA. More and comprehensive studies are needed to determine the effect of *T. gondii* infection on pathogenesis RA from other parts of the world.

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Conflict of interest

None

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