Original Article

Toxoplasmosis Frequency Rate in Rheumatoid Arthritis Patients in Northeastern Iran

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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.

Keywords:
Toxoplasmosis; Toxoplasma gondii; Rheumatoid arthritis; Seroprevalence; Patients

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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, severe clinical manifestations and even death have been observed in immunocompromised patients or during pregnancy (2).

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that 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 raise 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).

| 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% (*n*/n) 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.

| 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) |
| IgG | | | | |
| Positive | 24 (48.0) | 4 (10.0) | 28 (31.1) | <0.001 |
| Negative | 26 (52.0) | 36 (90.0) | 62 (68.9) |

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 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 academic | 14 (60.9%) | 16 (66.7%)| 30 (63.8%)|         |
| 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%)     | 6 (25.0%) | 8 (17.0%) |         |
|                            | 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 (AID). 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 (26).

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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