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The current status of *Toxoplasma gondii* infection among Egyptian rheumatoid arthritis patientsNagwa Mostafa El-Sayed<sup>1\*</sup>, Shereen Magdy Kishik<sup>2</sup>, Rasha Mohamed Fawzy<sup>3</sup><sup>1</sup>Medical Parasitology Department, Research Institute of Ophthalmology, Giza, Egypt<sup>2</sup>Parasitology Department, Faculty of Medicine, Benha University, Benha, Egypt<sup>3</sup>Rheumatology and Rehabilitation Department, Faculty of Medicine, Benha University, Benha, Egypt

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## ABSTRACT

**Objective:** To ascertain a relationship between *Toxoplasma gondii* (*T. gondii*) infection and rheumatoid arthritis (RA) disease among Egyptian patients.**Methods:** One hundred RA patients and 50 healthy subjects participated in this study. The patients were classified into three groups, namely G1, G2 and G3. Patients in G1 were recently diagnosed with RA with the disease duration of less than one year (prior treatment); G2 included RA patients receiving anti-tumor necrosis factor agents and RA patients in G3 received disease modifying anti-rheumatic drugs (methotrexate, antimalarial, corticosteroids). Serum samples of all participants were examined for the presence of anti-*Toxoplasma* immunoglobulin G (IgG) antibodies and positive samples were further analyzed for anti-*Toxoplasma* IgM antibodies to detect the possibility of reactivation of latent toxoplasmosis. Also, the association between *Toxoplasma* seropositivity and clinical, laboratory and radiological features of these patients were determined.**Results:** There was a significantly higher percentage of *T. gondii* IgG positivity in RA patients (54%) than in the controls (32%). At the same time, 20.40% of *T. gondii* IgG positive patients had anti-*T. gondii* IgM antibodies with a statistically significant difference as comparing to *T. gondii* IgG positive controls. Out of *T. gondii* seropositive patients, 20.37% had a lower IgG level with a mean titer of (65.3 ± 17.7) IU/mL, 46.29% had moderate level with a mean titer of (184.2 ± 60.0) IU/mL and 33.33% had higher level with a mean titer of (404.3 ± 50.0) IU/mL. A positive correlation was found between disease activity and *Toxoplasma* seropositivity. *T. gondii* seropositive RA patients had longer disease duration, longer time morning stiffness, higher numbers of tender and swollen joints and also increase in disease severity markers (erythrocyte sedimentation rate, C-reactive protein, disease activity score 28, anti-cyclic citrullinated peptide anti-bodies, rheumatoid factor) than *T. gondii* seronegative patients. As regards radiological findings, Larsen score was found significantly higher in *T. gondii* seropositive RA patients.**Conclusions:** The positive correlation between *T. gondii* infection and RA disease among Egyptian patients indicated the need to improve awareness of this parasitic infection and its management in this risk group.

## 1. Introduction

Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii* (*T. gondii*), an intracellular protozoan parasite. The infection is acquired by oral ingestion of the contaminated water or foods with *T. gondii* excreted in cat feces or undercooked meat containing

tissue cysts. In addition, this infection can be transmitted through the placenta, organ transplantation and blood transfusion[1]. *T. gondii* infection in immunocompetent individuals is mostly asymptomatic or can be manifested as lymphadenopathy and is usually followed by a lifelong latent infection that may be reactivated as a result of immune disorders inducing serious complications[2]. The prevalence of latent toxoplasmosis ranges from 20% to 80% among different populations, which depends on various sociological and environmental factors including hygienic standards, living habits and the number of cats in the environment, moisture and latitude[3].

Rheumatoid arthritis (RA) is a complex autoimmune disease affecting 1%–2% of the general worldwide population and more prevalent in females at late childbearing years of age[4]. The

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The study protocol was performed according to the Helsinki declaration and approved by the Research Ethics Committee, Faculty of Medicine, Benha University, Egypt. Informed written consent was obtained from all participants.

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etiopathogenesis of RA is multifactorial resulting from a complex interaction between genetic and environmental factors that can trigger the immune response[5]. This disease is characterized by an inflammatory synovitis, usually involving peripheral joints in a symmetric distribution causing cartilage damage, bone erosions and changes in joint integrity[6]. The patients may have a mild oligoarticular illness of brief duration with minimal joint damage. However, the most will progress to poly-arthritis with marked impairment[7].

The association between microbiological infections (bacterial, viral and parasite) and autoimmune disease has been shown in several studies. These infections are believed to act as triggers inducing or promoting autoimmune disease in genetically predisposed individuals by molecular mimicry, epitope spreading, or bystander activation[5,8,9]. Toxoplasmosis is considered as a major health problem in patients with systemic lupus erythematosus, systemic sclerosis, ankylosing spondylitis, autoimmune thyroid diseases and inflammatory bowel disease[10-12] and also its association with RA has been studied by several authors[7,8,13]. To ascertain a relationship between *T. gondii* infection and RA, this study was conducted to determine *T. gondii* positivity among Egyptian RA patients and its correlation with their clinical, laboratory and radiological findings.

## 2. Materials and methods

### 2.1. RA patients and controls

One hundred patients from the outpatient and inpatient clinics of the Rheumatology and Rehabilitation Department of Benha University Hospitals during the period from June 2015 to May 2016 were enrolled in this study. Seventy patients were female and thirty were male. The ages of the patients ranged from 30 to 58 years with a mean age of  $(43.1 \pm 4.8)$  years. All of them were subjected to full history taking, clinical examination and they met the classification criteria of the American College of Rheumatology/European League against Rheumatism for RA classification[14]. The patients were divided into three groups, G1, G2 and G3. G1 included 25 recently diagnosed RA patients with disease duration of less than one year (prior treatment). G2 included 25 RA patients receiving anti-tumor necrosis factor (TNF) agents (infliximab, etanercept and adalimumab) and G3 was formed by 50 RA patients receiving disease modifying anti-rheumatic drugs (DMARDS), methotrexate, antimalarial, corticosteroids.

Fifty control subjects from the general population of Benha City, Egypt were also enrolled in the study. They were 34 females and 16 males with a mean age of  $(40.3 \pm 1.9)$  years (ranged from 29–57 years) and were compatible with both sex and age of patients ( $P > 0.5$ ).

### 2.2. Investigations

#### 2.2.1. Laboratory investigations

All patients were investigated for erythrocyte sedimentation

rate (ESR) by Westergren method[15], C-reactive protein (CRP) and rheumatoid factor (RF) by latex agglutination tests (Leaner Chemicals Co., Spain), anti-cyclic citrullinated peptide antibodies (anti-CCP) by ELISA (Euro Diagnostica, Co., Sweden).

#### 2.2.2. Assessment of the disease activity score 28 (DAS28)

DAS28 is an index calculated after the examination of 28 swollen and tender joints involving hands, arms and knees. The DAS28 ranges from 1 to 9 where a low score ( $< 3.2$ ) indicates a low disease activity, a moderate score ( $3.2-5.1$ ) indicates moderate disease activity and a high score ( $> 5.1$ ) indicates high disease activity[16].

#### 2.2.3. Radiological examination

Plain X-ray (P–A views) on hands, wrists and feet were obtained and scored by Larsen score[17] that was applied for proximal interphalangeal joints (2–5), metacarpophalangeal joints (2–5) in each hand, four quadrants in both wrists and metatarso-phalangeal joints (2–5) in each foot. The grading scale ranged from 0 to 5 where 0 was intact bony outlines and normal joint space, 1 was erosion (diameter  $< 1$  mm), 2 referred to one or several small erosions (diameter  $> 1$  mm); 3 indicated marked erosion, 4 was severe erosion (usually no joint space left and the original bony outlines were only partly preserved) and 5 was mutilating changes (the original bony outlines have been destroyed). Then, the score was summed up giving a maximum score of 160 when all joints were fully destroyed.

#### 2.2.4. Determination of the anti-*T. gondii* antibodies positivity

Serum samples of all participants were analyzed for anti-*Toxoplasma* IgG antibodies using commercially enzyme immunoassay, *Toxoplasma* IgG kit, (DRG International Inc., USA). Anti-*T. gondii* IgG antibody levels were expressed as IU/mL, and a result equal or greater than 32 IU/mL was considered positive. Low IgG antibody level was less than 100 IU/mL, moderate level was 100–300 IU/mL while the high level was more than 300 IU/mL. Additionally, positive samples for anti-*T. gondii* IgG antibodies were further analyzed for anti-*T. gondii* immunoglobulin M (IgM) antibodies by the commercially enzyme immunoassay, *Toxoplasma* IgM kit, (DRG International Inc., USA). The tests were performed following the manufacturer's instructions.

### 2.3. Statistical analysis

Statistical analysis was carried out by SPSS software (statistical package for social science) (version 16, SPSS, Inc., USA). Qualitative data were expressed in numbers and percents and quantitative data were expressed as mean and standard deviation. The collected data were analyzed using *Chi*-square test, and student's *t*-test for significance differences.  $P < 0.05$  was considered statistically significant.

### 2.4. Ethical considerations

The study was approved by the Research Ethics Committee,

Faculty of Medicine, Benha University, Egypt. The aim of the study was explained to all participants, and an informed consent was obtained from all of them.

### 3. Results

The results revealed a higher *T. gondii* infection rate in RA patients (54%) as compared with the controls (32%), with a statistically significant difference ( $P = 0.01$ ). It was observed that 40% of the recently diagnosed RA patients (G1), 64% of patients treated with anti-TNF agents (G2) and 56% of patients treated with DMARDs (G3) had latent toxoplasmosis (Table 1).

**Table 1**  
*Toxoplasma* IgG positivity in RA patients versus healthy controls.

Groups	Anti- <i>Toxoplasma</i> IgG			
	IgG positive [n (%)]	IgG negative [n (%)]	Total	
RA patients	G1	10 <sup>b</sup> (40%)	15 (60%)	25
	G2	16 <sup>a</sup> (64%)	9 (36%)	25
	G3	28 <sup>a</sup> (56%)	22 (44%)	50
	Total	54 <sup>a</sup> (54%)	46 (46%)	100
Controls	16 (32%)	34 (68%)	50	

<sup>a</sup>: Significant difference compared with the controls; <sup>b</sup>: No significant difference compared with the controls.

Additionally, *T. gondii* IgG positive sera of the participants were further analyzed for the presence of anti-*T. gondii* IgM antibodies to detect the possibility of reactivation of latent toxoplasmosis (Table 2). It was found that 20.40% of *T. gondii* IgG positive patients had anti-*T. gondii* IgM antibodies with a statistically significant difference ( $P = 0.049$ ) as comparing to *T. gondii* IgG positive controls. Out of them, 31.25% of patients treated with anti-TNF agents (G2) and 21.40% of patients treated with DMARDs (G3) had a significant reactivation of latent toxoplasmosis ( $P = 0.014$ ,  $P = 0.046$ , respectively).

**Table 2**  
*Toxoplasma* IgM positivity among *Toxoplasma* IgG positive RA patients.

Group	Anti- <i>Toxoplasma</i> IgM			
	IgM positive [n (%)]	IgM negative [n (%)]	Total	
RA patients	G1	0 <sup>b</sup> (0.00%)	10 (100.00%)	10
	G2	5 <sup>a</sup> (31.25%)	11 (68.75%)	16
	G3	6 <sup>a</sup> (21.40%)	22 (78.60%)	28
	Total	11 <sup>a</sup> (20.40%)	43 (79.60%)	54
Controls	0 <sup>b</sup> (0.00%)	10 (100.000%)	16	

<sup>a</sup>: Significant difference compared with the controls; <sup>b</sup>: No significant difference compared with the controls.

Another interesting finding was the significantly higher levels of anti-*T. gondii* IgG in RA patients than in controls ( $P = 0.0001$ ). Out of 54 *T. gondii* IgG positive patients, 20.37% had a lower IgG level with a mean titer of ( $65.3 \pm 17.7$ ) IU/mL, 46.29% had moderate level with a mean titer of ( $184.2 \pm 60.0$ ) IU/mL and 33.33% had higher level with a mean titer of ( $404.3 \pm 50.0$ ) IU/mL. However, the most of *T. gondii* IgG positive controls had lower level with a mean titer of ( $55.2 \pm 16.0$ ) IU/mL and only 25.00% of them with a moderate level ( $121.0 \pm 20.6$ ) and none of them had a higher level (Table 3).

*T. gondii* seropositive RA patients had longer disease duration than *T. gondii* seronegative patients in spite of this difference did not

reach to any significant value. Additionally, *T. gondii* seropositive RA patients had longer time morning stiffness, higher numbers of tender and swollen joint and also increase in disease severity markers, ESR, CRP, DAS28 score, anti-CCP and RF, than those of *T. gondii* seronegative patients. The difference between both groups was statistically significant. As regards radiological findings, Larsen score was found significantly higher in *T. gondii* seropositive RA patients (Table 4).

**Table 3**

Sero-intensity of *Toxoplasma* IgG antibodies among *Toxoplasma* positive RA patients and the controls.

Anti- <i>Toxoplasma</i> IgG level (IU/mL)	RA patients (n = 54)			Controls (n = 16)	
	G1 (n = 10)	G2 (n = 16)	G3 (n = 28)		
Low (< 100)	Number	8 <sup>a</sup>	0 <sup>a</sup>	3 <sup>a</sup>	12 (75%)
	Total [n (%)]	11 (20.37%)			
	Mean titer (IU/mL)	65.3 $\pm$ 17.7			55.2 $\pm$ 16.0
Moderate (100–300)	Number	2 <sup>a</sup>	9 <sup>a</sup>	14 <sup>a</sup>	4 (25%)
	Total [n (%)]	25 (46.29%)			
	Mean titer (IU/mL)	184.2 $\pm$ 60.0			121.0 $\pm$ 20.6
High (> 300)	Number	0 <sup>a</sup>	7 <sup>a</sup>	11 <sup>a</sup>	0 (0%)
	Total [n (%)]	18 (33.33%)			
	Mean titer (IU/mL)	404.3 $\pm$ 50.0			-

<sup>a</sup>: Significant difference compared with the controls.

**Table 4**

Correlation between *Toxoplasma* seropositivity and clinical, laboratory and radiological findings of RA patients.

Clinical, laboratory and radiological findings	RA patients (n = 100)		P
	<i>T. gondii</i> seropositive (n = 54)	<i>T. gondii</i> seronegative (n = 46)	
Disease duration (years)	10.10 $\pm$ 3.21	8.50 $\pm$ 6.28	0.1040
Morning stiffness (min)	70.00 $\pm$ 13.2 <sup>a</sup>	62.00 $\pm$ 22.40	0.0290
Swollen joint count	8.21 $\pm$ 4.56 <sup>a</sup>	6.31 $\pm$ 1.12	0.0070
Tender joint count	6.51 $\pm$ 4.08 <sup>a</sup>	4.34 $\pm$ 2.52	0.0020
DAS28	3.60 $\pm$ 0.30 <sup>a</sup>	2.20 $\pm$ 0.60	< 0.0001
Laboratory parameters			
RF (IU/mL)	Positivity	83%	78%
	Mean titer	138.00 $\pm$ 64.60 <sup>a</sup>	64.00 $\pm$ 6.60
Anti-CCP (IU/mL)	Positivity	72%	64%
	Mean titer	80.00 $\pm$ 20.40 <sup>a</sup>	68.00 $\pm$ 18.60
ESR (mm/h)	72.40 $\pm$ 16.4 <sup>a</sup>	57.70 $\pm$ 13.40	< 0.0001
CRP (mg/L)	12.30 $\pm$ 1.30 <sup>a</sup>	9.70 $\pm$ 1.20	< 0.0001
Radiological findings			
Larsen score	54.00 $\pm$ 8.70 <sup>a</sup>	37.00 $\pm$ 6.30	< 0.0001

<sup>a</sup>: Significant correlation between *Toxoplasma* seropositive and clinical, laboratory and radiological findings of RA patients as compared with seronegative patients.

### 4. Discussion

Human toxoplasmosis is considered as a major health problem among various Egyptian patients with a prevalence of 59.10% among ocular patients[18], 56.70% among schizophrenia patients, 40.00% among patients with depressive disorder[19] and 33.67% among blood donors[1]. Thus, this study determined the relationship between *Toxoplasma* infection and Egyptian RA patients by detecting IgG and IgM antibodies in blood samples of the patients compared with the controls. The diagnosis of toxoplasmosis might be highly desirable in those patients as they may be at risk for reactivation of latent infection as well as increase the risk of acute

toxoplasmosis.

Egyptian RA patients were found to have a significant latent toxoplasmosis (54%) with elevated levels of IgG to *T. gondii* compared to normal controls (32%) and also the presence of *Toxoplasma* IgM antibodies in 20.4% of *T. gondii* IgG positive patients indicated the reactivation of *T. gondii* infection in them, especially those receiving anti-TNF and DMARDs treatments. These results are compatible with Kuba *et al.*[7] who found that the seroprevalence of *T. gondii* IgM and IgG were 20.40% and 33.33% in RA patients receiving methotrexate, 8.00%, and 36.00% in RA patients without treatment, while it was 24.00% and 12.00% in healthy controls. In Kirkuk, a city in Iraq, the overall seroprevalence of toxoplasmosis was observed in 54.09% RA patients, and 47.54% and 6.55% of them had *Toxoplasma* IgG and IgM antibodies, respectively[13]. Furthermore, *Toxoplasma* positivity was found in 63% of European RA patients[8]. Shapira *et al.*[11] showed that anti-*T. gondii* IgG were positive in 42% of patients with various autoimmune diseases versus 29% of controls. Conversely, Sert *et al.*[20] found no significant difference between the frequency of IgG antibodies positivity and IgG levels in the patients with reactive arthritis and those in the healthy asymptomatic controls (52.0% vs. 47.5%) and they suggested that *T. gondii* does not seem to be a triggering agent for reactive arthritis and past infection may be a coincidental finding.

Infection with *T. gondii* results in a strong and persistent T-helper-1 (Th1) response characterized by the production of pro-inflammatory cytokines, including interleukin-12, interferon- $\gamma$  and TNF- $\alpha$ . The combined action of these cytokines and other immunological mechanisms protect the host against rapid replication of *Toxoplasma* tachyzoites and subsequent pathological changes. In chronic toxoplasmosis, depletion of these cells in the patients receiving immunosuppressive drugs can cause reactivation of this latent infection and may result in severe complications[21-23]. Despite the effect of DMARDs, especially methotrexate, to reverse the symptoms of RA disease, reduce the progression of joint damage and improve the quality of life of patients[24], they are also used for long-time decreasing immune response and promoting the microbial infections[25]. In this study, it was found that 56.0% of RA patients receiving methotrexate had chronic toxoplasmosis and 21.4% of them had reactivation of this latent infection. This finding was in agreement with van der Veen *et al.*[26] who reported that RA patients receiving methotrexate are more liable to have infections of the respiratory tract and skin and to receive prescriptions for antibiotics than RA patients receiving no DMARDs other than methotrexate.

In addition, the use of anti-TNF agents such as infliximab, etanercept and adalimumab have a number of contraindications and side effects, including reactivation of opportunistic infections such as toxoplasmosis[27,28]. In the present study, it was observed the increased risk of toxoplasmosis in RA patients treated with anti-TNF- $\alpha$  therapy (64.00%) and half of them (31.25%) had reactivation of this disease. This observation was in agreement with Lassoued *et al.*[21] who reported two cases of ocular toxoplasmosis in patients with rheumatoid arthritis receiving anti-TNF- $\alpha$  agent.

One of the two cases was because of the reactivation of previous toxoplasmosis infection, whereas the other had primary acquired ocular toxoplasmosis. Also, cerebral toxoplasmosis has been reported in RA patients receiving methotrexate, adalimumab and infliximab[22,23]. Experimentally, El-Sayed *et al.*[28] concluded that etanercept, a TNF- $\alpha$  antagonist, played a role in reactivation of latent toxoplasmosis by increasing the number and size of tissue cysts in brain of *T. gondii* infected mice. Treatment with immunosuppressive drugs stimulated the release of bradyzoites from tissue cysts which convert into tachyzoites and proliferate in host tissue without restriction, leading to the dissemination of *Toxoplasma* organisms to other cells. Trimethoprim-sulphamethoxazole prophylaxis was recommended to be given to patients who needed high doses of immunosuppressive to prevent toxoplasmosis, especially if there is serologic evidence of latent infection[29].

It is important to point out that a significant correlation was found between RA disease severity and *Toxoplasma* positivity. *Toxoplasma* seropositive RA patients presented with severe clinical manifestations such as morning stiffness, higher numbers of tender and swollen joints and higher DAS28 scores than *Toxoplasma* seronegative patients. The disturbances in the immunological response interfere with parasite control and thereby have an impact on the clinical course. RA patients typically have circulating auto-antibodies, rheumatoid factor and anti-cyclic citrullinated peptide[30,31]. These antibodies were significantly linked to *Toxoplasma* positivity, particularly when presenting in high titres. The obtained results were supported by several clinical and experimental studies which found that microbial infections can induce and/or exaggerate the symptoms of arthritis[5].

There might be a causal relationship between *Toxoplasma* infection and RA. Either, RA-susceptible genetic and environmental factors, such as predisposed genes and living habits, may cause increased risk of infection even before or in the early stage of RA or RA-associated abnormal immune response and immunosuppressive medicine may contribute to decreased host defense to infection[32]. On the other hand, *Toxoplasma* infection may be a risk factor for the development of RA. It was reported that *Toxoplasma* may cause a symmetrical polyarthritis of the small joints of hands, wrists and knees in a rheumatoid pattern[33,34]. Different parasites can cause joint diseases by several mechanisms including invasion from neighboring bones or muscles via the blood or lymphatic with the presence of the parasitic stages in the joint cavity and also by triggering a reactive inflammatory reaction to the presence of the parasite in the surrounding tissue without an actual joint invasion[35].

Based on the results obtained, the positive correlation between *T. gondii* infection and RA disease among Egyptian patients indicated the need to improve the awareness of this parasitic infection and its management in this risk group to avoid any resulting serious complications from reactivation of a latent infection.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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