

## Original paper

# Relationship between *Toxoplasma gondii* and Autoimmune Disease in Aborted Women in Najaf Province

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## Abstract

**Background:** Autoimmune diseases are chronic conditions initiated by the loss of immunological tolerance to self-antigens. The pathogenic hypothesis comprises a complex interaction between genetic, environmental and hormonal factors. Aim of this study to assess the association between toxoplasmosis and some autoimmune diseases.

**Methods:** A case-control study was conducted to estimate the association between toxoplasmosis and some of autoimmune disease including diabetes mellitus, rheumatoid arthritis and systemic lupus erythematosus. The present study was carried out on 48 aborted women, and 21 non-aborted women, all of these cases were limited to females only in the reproductive age (16-50 years). Toxo-IgM and Toxo-IgG antibodies were detected with (ELISA) (Human/ Germany) in both groups, and measure blood sugar, ACCP, and ANF.

**Results:** The infections with toxoplasmosis were 5 (17.9%) and 23 (82.1%) among those with diabetes mellitus and rheumatoid arthritis disease respectively. However, none of the 8 women with SLE had toxoplasmosis, Furthermore; the results revealed that toxoplasmosis positive women were about 4.4 folds more likely to have RA. (OR= 4.4, P≤ 0.01).

**Conclusion:** This research concludes that the primary or secondary infections with *Toxoplasma gondii* can mimicry immune response and make shift in the immune response to induce autoimmune disease.

**Keywords:** *Toxoplasma gondii* and autoimmune disease, *Toxoplasma gondii*, Rheumatoid arthritis.

## Introduction

The autoimmune diseases result from inappropriate responses of the immune system to self-antigens. The etiology of autoimmune diseases remains largely unknown but etiologic factors include genetic abnormalities and infections<sup>(1)</sup>.

The protozoan parasite *Toxoplasma gondii* (*T. gondii*) which causes toxoplasmosis, There are three infective stages of *T. gondii*, a rapidly dividing invasive called tachyzoite, and a slowly dividing bradyzoite in tissue cysts, and an environmental stage, called the sporozoite, protected inside an oocyst<sup>(2)</sup>.

Tachyzoites are the dissemination form; they are able to invade virtually all cell types, where they multiply in a parasitophorous vacuole. Tachyzoites conversion into slow-dividing stage bradyzoites and form tissue cysts<sup>(3)</sup>.

The parasite has also been implicated in a number of autoimmune disorders including autoimmune thyroid diseases, systemic sclerosis<sup>(4,5)</sup> rheumatoid arthritis, inflammatory bowel disease<sup>(6)</sup>, possibly related to host/pathogen antigen homology<sup>(7,8)</sup>.

There are multiple mechanisms by which the pathogen can lead to autoimmunity, that include 1- Molecular mimicry i.e. immunological cross-reactivity between

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the parasite and components of host tissues<sup>(9, 10)</sup>. 2-Bystander activation would imply that the inflammatory setting and the paracrine secretion of T cell growth factors induce the expansion of activated auto reactive T cells, whose small number was previously insufficient to drive an autoimmune disease<sup>(11)</sup>. 3-Epitope spreading Pathogen induced tissue inflammation may result in local activation of APC and enhanced processing / presentation of self-antigens that causes T cell priming, followed by T cell activation and expansion of additional specificities<sup>(12, 13)</sup>. So the aim of the study to evaluate if *Toxoplasma gondii* is trigger to develop autoimmune diseases including diabetes mellitus, rheumatoid arthritis and systemic lupus erythematosus.

## Methods

**Study design:** This case-control study was performed on 69 women, the mean age of the participant women were  $29.5 \pm 7.6$  (range: 16-50) years. The women in the current study were classified into two groups; First group: involved 48 women with bad obstetrician history (BOH), who suffered from previous abortions, Second group: included 21 non aborted women without obstetric problems and had more than one successful birth. They were attending AL-Zahraa Maternity and Pediatric Teaching Hospital.

This study was approved before its commencement by the ethical committee of the Faculty of Medicine, University of Kufa. And informed consent was obtained from all individuals.

**Sample collection:** Five milliliter of venous blood was collected from 48 patients and 21 control they were attending AL-Sader medical city and AL-Hakeem Hospital in Najaf province in the period from March 2013 to March 2014. In sterile serum tube and left for one hour at room temperature to allow the clot to form. Then, centrifuged at 3000 rpm for 15 min. to separate the serum which divided in

ependorf tubes (200 $\mu$ l) and kept at deep freeze (-20 C°) until used.

**Immunological tests:** The search for anti-*T. Gondii* antibodies in serum was performed by Enzyme Linked Immunosorbent Assay (ELISA) by using ToxoIgM ELISA and Toxo-IgG ELISA (Human-Germany). It was done according to manufacturer's instructions.

Measure glucose in serum by using kit (Randox-UK) and reading the results in spectrophotometer. It was done according to manufacturer's instructions as the following. 1ml of glycerin was added to gain tube, then added 10 ul of serum and waiting 10min. Reset device by distilled water and pressed to zero. Then sample was transferred from gain tube to cuvette was inserted into the spectrophotometer and pressed read within 60 min. The WHO diagnostic criteria for diabetes should be maintained - fasting plasma glucose  $\geq 7.0$ mmol/l (126mg/dl) or 2hours after meal random plasma glucose  $\geq 11.1$ mmol/l (200mg/dl)<sup>(14)</sup>.

Enzyme immunoassay for detection of IgG antibodies to specific cyclic citrullinated peptides (ACCP) (Aeskutiza Diagnostics-Germany). Which has high sensitivity and specificity in detecting antibodies in patients with RA. It was done according to manufacturer's instructions. In addition to the diagnostic criteria of rheumatoid arthritis (RA) that are consistent with the 2010 American College of Rheumatology including the following clinical features: Inflammatory arthritis involving three or more joints, positive rheumatoid factor (RF) and/or anti-citrullinated peptide/protein antibody, elevated levels of C-reactive protein (CRP) or the erythrocyte sedimentation rate (ESR). The duration of symptoms is more than six weeks.

Antinuclear antibodies factor (ANF) test associated with systemic lupus erythematosus (SLE) (Human- Germany). The ANF test provides mean of detecting anti-DNP (deoxyribonucleoprotein) in human serum. SLE Latex reagent is a stabilized buffered suspension of

polystyrene latex particles that have been coated with DNP. When the latex reagent is mixed with the serum containing antibodies to DNP agglutination occurs. In addition to the diagnostic criteria for lupus, by the American College of Rheumatology (ACR). at least four of these criteria should be present, Malar rash—a rash over the cheeks and nose, often in the shape of a butterfly, photosensitivity—a reaction to sun or light that causes a skin rash to appear or get worse, oral ulcers—sores appearing in the mouth, arthritis –joint pain and swelling of two or more joints in which the bones around the joints do not become destroyed, kidney disorder including persistent protein or cellular casts in the urine, neurological disorder including seizures or psychosis, blood disorder— anemia (low red blood cell count), leukopenia (low white blood cell count), or thrombocytopenia (low platelet count), immunologic disorder—anti-DNA or positive anti- phospholipid antibodies (Abnormal antinuclear antibody ANA).

**Statistical analysis:** Data of the studied group were checked for any error or inconsistency, entered and analyzed by using the statistical package for social sciences (SPSS) version 22, 2014. Chi square test was used to compare

frequencies. Level of significance, P.value, was tailed in all comparison and set at  $\leq 0.05$  to be considered as significant difference or correlation.

## Results

The results of present study showed that 17(24.6%), 44(63.8%) and 8(11.6%) were patients with diabetes mellitus, rheumatoid arthritis and SLE respectively. The seroprevalence toxoplasmosis were 5(17.9%) and 23(82.1%) among those with diabetes mellitus and rheumatoid arthritis disease respectively. However, none of the 8 women with SLE had toxoplasmosis, Furthermore, the results revealed that toxoplasmosis positive women were about 4.4 folds more likely to have R. A. (OR= 4.4,  $P \leq 0.01$ ) table (1).

From other point of view, it had been found the occurrence D.M, R.A. and SLE were 76.5%, 68.2% and 62.5 among aborted women respectively. D.M, R.A. and SLE were 23.5%, 31.8% and 37.5 respectively among non-aborted women, in addition, this table is shown patients with D.M. were risk for occurrence abortion but not reach to statistical significant (OR=1.6  $p \leq 0.5$ ), table (2).

**Table 1.** Distribution of *Toxoplasma* prevalence with chronic disease DM, RA and SLE

Groups	Toxo-Positive		Toxo-Negative		Total		OR (CI 95%)	P
	No.	%	No.	%	No.	%		
Patients with DM	5	17.8	12	29.2	17	24.6	0.5 (0.2-1.7)	0.72
Patients with R.A	23	82.1	21	51.2	44	63.7	<b>4.4</b> <b>(1.4-13.8)</b>	<b>0.01</b>
Patients with SLE	0	0	8	19.5	8	11.5	0 (0.004-1.2)	0.07
Total	28	100	41	100	69	100		

**Table 2.** Distribution of studied groups by their chronic disease with DM, RA and SLE.

Disease	abortion						OR (CI 95%)	P
	aborted		Non aborted		Total			
	No.	%	No.	%	No.	%		
DM	13	27.0	4	19	17	24.6	1.6 (0.4- 5.6)	0.5
RA	30	62.5	14	66.6	44	63.7	0.8(0.3 - 2.5)	0.7
SLE	5	10.4	3	14.2	8	11.5	0.7(0.2 - 3.4)	0.6
Total	48	100	21	100	69	100		

## Discussion

Statistical analysis showed that patients with toxoplasmosis were about 4.4 fold more likely to have RA (OR=4.4, P=0.01). Other studies were support these findings, <sup>(15)</sup> pointed that, a possible link between exposure to *T. gondii* infection and RA. <sup>(16)</sup> Concluded the higher prevalence of anti-Toxoplasma-antibodies (IgG) were associated with in European RA patients (p<0.05), and concluded *Toxoplasma gondii* playing a role in pathogenesis of several autoimmune diseases like rheumatoid arthritis. Whereas <sup>(17)</sup> mentioned that, RA patients treated with methotrexate (MTX) will under the risk of toxoplasmosis infection, and this association may due to MTX help in blocking the production of TNF- $\alpha$ , which play very important role in the immune response against *T. gondii* infection and other pathogenic infection <sup>(18, 19)</sup> recorded that, *Toxoplasma gondii* may cause a symmetrical polyarthritis of the small joint of hands, wrists and knee in a rheumatoid pattern. And he noticed all patients with RA have serological evidences of acute toxoplasma infection. Moreover, Anti-T.gondii-antibodies (IgG) present in 63% of RA patients (p<0.0001) <sup>(20)</sup>. The results of the current study are disagreement with the study by <sup>(21)</sup> who reported that infection with *Toxoplasma gondii*, is capable of ameliorating the spontaneous development of arthritis in mice, and indicating that IL-10 production from DC of *T. gondii*-infected mice may down regulate the spontaneous development of arthritis that is known to be IL-17 dependent <sup>(22)</sup>, and this consistent with <sup>(23)</sup> who said that, IL-10 suppresses IL-17 expression <sup>(24)</sup>. The results by A'aiz (2010) <sup>(25)</sup> revealed that, among 38 patients with toxoplasmosis only 4 (10.5%) showed positive results for RF and concluded that there is no correlation between toxoplasmosis and RF. Other studies revealed that, increase in serum level of IL-17 during initial stages of

infection with *Toxoplasma gondii*, which IL-17 was involved in the development and early recruitment neutrophils, which are essential to clear the parasites <sup>(26; 27)</sup>. So the association between infection with *Toxoplasma gondii* and rheumatoid arthritis is possible because IL-17 has important role in development RA. Seventeen patients with diabetes mellitus were examined for the presence of *Toxoplasma* antibodies. Among patients, 5 cases (17.9%) were seropositive and 12 patients (29.3%) were seronegative. These results consistent with <sup>(28)</sup> among 205 diabetic patients, 60 cases (29.3%) were seronegative and 145 patients (70.7%) were seropositive. <sup>(29)</sup> Revealed that, pregnant women with latent toxoplasmosis had significantly higher blood glucose levels. The results by <sup>(30)</sup> showed that the total toxoplasmosis samples positive ELISA antibody IgG (51.4%) and antibody IgM (% 11.4) among diabetes mellitus. <sup>(31)</sup> Reported that *Toxoplasma gondii* plays an important role in the pathogenesis of both types of diabetes

Eight patients with lupus were examined for the presence of *Toxoplasma* antibodies, all patients were seronegative. <sup>(32)</sup> Showed that, some infectious agents, such as *Toxoplasma gondii* may have a protective effect against SLE. Whereas study by <sup>(33)</sup> showed that revealed that T. gondii seropositivity rates in SLE patients were (60 %). <sup>(34; 35)</sup> revealed that seropositivity for *Toxoplasma gondii* is more common among SLE patients than control individuals. Likewise <sup>(36)</sup> recorded that; seropositive of toxoplasmosis were 36% among SLE patients.

## Conclusion

This research conclude that the primary or secondary infection with *Toxoplasma gondii* can mimicry immune response and make shift in the immune response to induce autoimmune disease.

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