Kirkuk Journal of Medical Sciences

ORIGINAL ARTICLE

Detection of *Toxoplasma gondii* IgM in a Sample of Rheumatoid Arthritis Patients Receiving Biological Therapy

Ikram Khazal Qasim Al- hasso [1,*], Saeed Hameed Hussein [1], Asmaa Zaki Shetawi [1]

[1]*Department of Microbiology, College of Medicine, University of Mosul, Mosul, IRAQ.*

[*]*Corresponding author email: ikramhassow@uomosul.edu.iq*

Received: 05 May 2024
Accepted: 03 August 2024
First published online: 15 February 2025

DOI: 10.32894/kjms.2024.149466.1105.

ABSTRACT

Background: *Toxoplasma gondii* is an intracellular protozoan coccidian parasite, that can establish a widespread infection in people with autoimmune disorders. Rheumatoid arthritis is an autoimmune inflammatory disorder in which biological therapy is one of the treatment modalities. This study aims to evaluate *Toxoplasma gondii* IgM antibody seropositivity in Rheumatoid arthritis patients in Mosul city who were under biological therapy.

Methods: A case control study was conducted at Mosul City in Iraq, enrolled 190 participants who were divided into 2 groups, 95 patients had RA on biological therapy and the other 95 participants were a healthy control group. Using the enzyme-linked immunosorbent assay technique, anti-*Toxoplasma* IgM antibodies have been identified in the sera of ninety-five rheumatoid arthritis patients undergoing biological therapy for six months, as well as in the serum of ninety-five healthy controls.

Results: Anti-*Toxoplasma* IgM antibodies were detected in (17%) of RA patients and only (2.1%) of controls (P=0.0008). The mean value of IgM antibody was 29.232 IU/mL in rheumatoid patients versus 14.59 IU/mL in controls (P=0.0463). The highest Toxoplasma IgM antibody level was in the age group (31-49 years) with (P=0.0145). There was no significant connection between seropositivity and the three types of biological therapy (P=0.389).

Conclusion: There is an association between long-term biological treatments for RA and the onset of toxoplasmosis.

Key words: Toxoplasmosis; Rheumatoid Arthritis; IgM antibody; ELISA; Autoimmunity.

INTRODUCTION

Rheumatoid arthritis (RA) is a prevalent type of inflammatory arthritis, it is a long-term immune-mediated illness that causes chronic synovial inflammation and progressive joint deterioration, resulting in disability and loss of life [1, 2].

Treatment purposes in RA include lowering articular inflammation, relieving discomfort, minimizing joint destruction, and preserving physical function, it has been found that tumor necrosis factor (TNF) antagonists and further biological disease-modifying antirheumatic medications (DMARDs) have improved therapy of RA during the last two decades and that biological therapies have dramatically revolutionized RA treatment, enabling for specific elimination of essential inflammatory components that leads to RA development [3, 4].

The conventional autoimmune disease therapeutic techniques are ineffective and the main aim of treatment is the targeting of cytokines and cells involved in the inflammatory process and hence minimize immune activation and inflammatory damage [5, 6].

Biological medications commonly used in RA treatment included etanercept which is a TNF-receptor fusion molecule besides infliximab which is a chimeric monoclonal antibody as well as adalimumab, a human monoclonal antibody. It has been shown that these biological medications are connected with a rise in the extent of severe infections in order of 6 per thousand patients who were managed yearly [3, 7].

Toxoplasma gondii (*T. gondii*), is a zoonotic parasitic protozoan that resides within the host's cells and causes Toxoplasmosis and it may invade and multiply in nucleated cells. It is a prevalent illness that have major health consequences, and affects one-third of the global population [8]. This parasitic disease has an inflammatory feature in which the T-helper lymphocytes' immune response as well as the pro-inflammatory mediators will contribute to the pathogenesis of this disease. Humoral immune response in the form of specific anti-Toxoplasma antibody formation will be detected throughout the infection [9]. With the advancement of understanding of *T.gondii* and its association with the immune system, it has recently been established that Toxoplasmosis may contribute to the occurrence of numerous autoimmune illnesses via immunological cross-reactivity between the parasite and host tissue components [10]. Given that the parasitic *T. gondii* infection is more frequent in the immunocompromised individuals, there is a growing awareness in establishing if there is a link between *T. gondii* infestation and the development of autoimmune diseases such as RA [11–13].

An immunocompetent host may experience a self-limiting febrile illness when infected by the parasitic disease *Toxoplasma gondii*, but in the immunocompromised individuals such as those who have rheumatoid arthritis and taking immunosuppressing medications, this infection can be devastating. Immunosuppression is well-known cause of predisposing patients to severe forms of Toxoplasmosis; and severe cases have been reported in patients who take immunosuppressive treatment for inflammatory disorders and autoimmune disease as rheumatoid arthritis [14].

For the first time in Mosul city, the current study aimed, to assess *T. gondii* infection amongst individuals suffer from rheumatoid arthritis and the effect of this autoimmune disease in increasing the susceptibility to this parasitic infection Toxoplasmosis as the taking of biological medications will lower the immunity of the patients making them more prone to toxoplasmosis. The aim of the current study was to assess the seropositivity of IgM antibodies against *Toxoplasma gondii* in patients with rheumatoid arthritis in Mosul City receiving biological treatment.

MATERIAL AND METHODS

A total of 190 patients were included in this case control study conducted at the Rheumatology Clinic of Al-Salam Hospital, located in Mosul, Iraq. The study commenced in March 2022 and concluded in November 2022. Participants were divided into two groups. The first group consisted of ninety-five patients diagnosed with rheumatoid arthritis (RA), who were undergoing treatment with biological tumor necrosis factor (TNF) inhibitors, specifically etanercept, infliximab, and adalimumab. The second group comprised ninety-five healthy persons considered as a control group. Within the initial group, forty-four patients received infliximab therapy with an average treatment duration of 5.1±1.5 months, thirty patients were administered etanercept with an average duration of 6.5±1.3 months, and twenty-one patients were treated with adalimumab, with a mean duration of 4.7±2.2 months.

Three milliliters of blood per participant were collected in EDTA tubes, and centrifuged, serum stored at -20°C until analysis. ELISA was used to detect anti-Toxoplasma IgM antibodies, following the protocol by DRG International, Inc. (Catalogue Number: EIA-3520). Values over 28 IU/mL indicated positive Toxoplasmosis status.

Statistical analysis was performed using SPSS (version 24.0) and GraphPad Prism (version 9.0.0). Data frequency was shown as a percentage. Chi-square and Fisher's exact tests examined non-parametric data for significant differences,

Table 1. Sociodemographic values of the studied groups

Variable	Patients with RA (N = 95)	Control (N = 95)	P-Value
Age (Mean ± SD)	40.1 ± 9.7 Years		
Age Groups (Years)			
< 30	18 (18.9%)	20 (21.1%)	0.114
31-49	54 (56.9%)	51 (53.7%)	
≥ 50	23 (24.2%)	24 (25.3%)	
Disease Duration	8.2 ± 2.5 Years		
DAS-28 (Mean ± SD)	5.37 ± 3.18		
Gender			
Female	87 (91.6%)	89 (93.7%)	0.208
Male	8 (8.4%)	6 (6.3%)	
ESR (Mean ± SD)	47 ± 18.41 mm/hr		
CRP of RA Patients	Positive: 89 (93.7%)	Negative: 6 (6.3%)	
Rheumatoid Factor (RF)	Positive: 82 (86.3%)	Negative: 13 (32.6%)	

SD: Standard deviation, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, DAS: Disease activity index.

with p-values equal and below 0.05 deemed statistically significant.

RESULTS

Ninety-five patients with RA who underwent biological therapy were included in this study. Among these patients, 87 (91.6%) were female, and 8 (8.4%) were male. The mean age of the patients was 40.1 years, with a standard deviation of 9.7 years. Detailed demographic data are provided in Table 1.

The findings revealed an elevated *T. gondii* antibody prevalence among patients with RA, with sixteen (17%) RA patients demonstrating antibodies compared to only two (2.1%) patients in the control group (P-value = 0.0008). The relative risk is calculated at 1.93, accompanied by a 95% confidence interval ranging from 1.42 to 2.37, as illustrated in Figure 1.

The IgM descriptive statistics in IU/mL for the studied groups, presented in Figure 2, exhibit a statistically significant difference.

In the group of arthritis patients, the mean age was 40.1±9.7 years, and the average duration of the disease was 8.2±2.5 years. The seropositivity for anti-toxoplasma IgM antibodies was found to be significantly more prevalent in females 15 (93.8%) compared to males 1 (6.2%), with a statistically significant difference observed in the gender distribution of arthritis patients (*P*=0.0001). The gender differences are illustrated in Figure 3.

Considering the seropositivity of *Toxoplasma gondii* IgM antibodies by age category, anti-Toxoplasma IgM antibody seropositivity is more common in age group (31-49) years

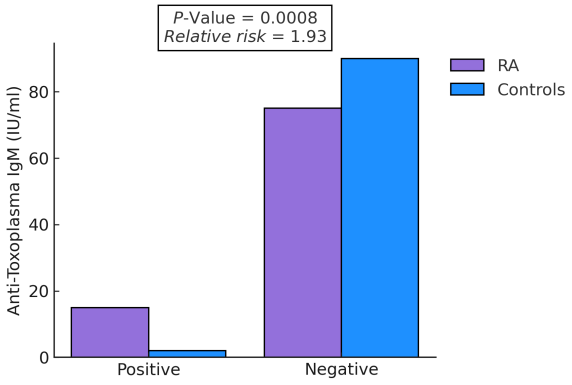

Figure 1. *Toxoplasma* IgM antibody seropositivity in RA patients versus controls

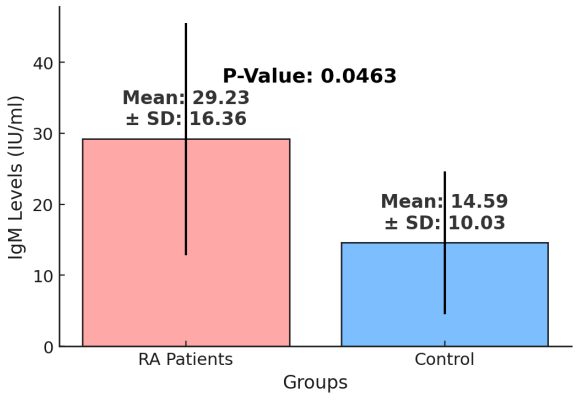

Figure 2. Comparison of IgM mean Levels in Rheumatoid Arthritis Patients and Controls

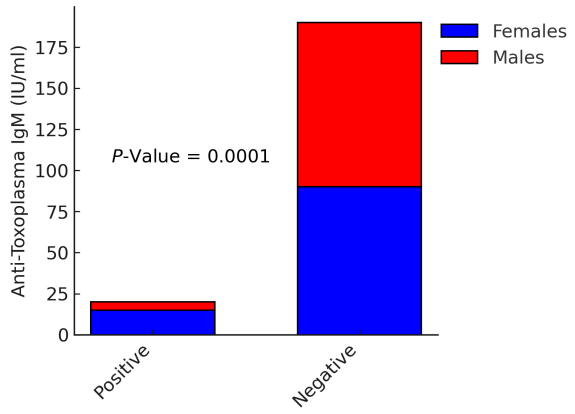

Figure 3. *Toxoplasma* IgM antibody seropositivity in RA cases regarding sex distribution

patients who had the uppermost ratio of *T. gondii* antibodies 7 (43.7%), while 5 patients were seropositive (31.2%) in the age group (< 30 years) and 4 (25%) in the age group (≥ 50 years) with a significant variation and (*P*=0.0145). Regarding biological treatment received and antibody seropositivity, it was shown that there are no statistically significant variations regarding the three types of biological therapies and antibody positivity (*P*=0.389, χ^2 = 1.884) as shown in Figure 4.

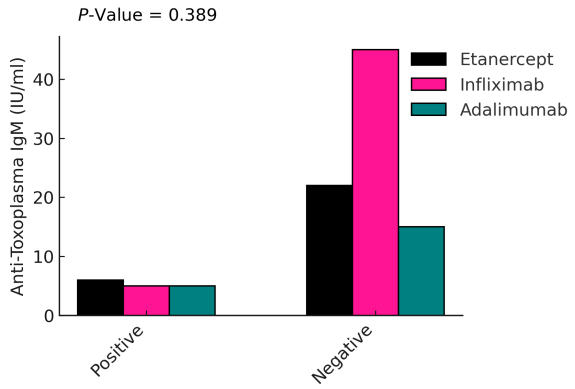

Figure 4. *Toxoplasma* IgM antibody seropositivity in RA cases regarding biological therapy

DISCUSSION

The worldwide intracellular parasite *Toxoplasma gondii* is the cause of the zoonotic disease toxoplasmosis. It may increase the chance of developing auto-immune conditions, such as rheumatoid arthritis [14].

The present study showed a significant relationship between *Toxoplasma* IgM antibodies and rheumatoid arthritis patients who receive biological therapy in Mosul city, Iraq, so it is critical to raise awareness about this parasitic infection and its treatment strategies in this highly risk group patients. The major approach for detecting *T. gondii*-specific antibodies, which suggests earlier exposure, is serological tests, the existence of Toxoplasmosis IgM antibodies represents an acute infection, as a result, these patients are required to be closely evaluated and treated in this regard [15].

Since toxoplasmosis is risky in patients with RA, reactivation of toxoplasma cysts during immunosuppressive medication treatment has serious consequences, including toxoplasma encephalopathy and even activation of immune modulators [16]. Furthermore, these patients' use of immunosuppressive medications inhibited TNF-secretion; a Th1 responsive mediator involved in the protection against acute and chronic toxoplasmosis [17].

Recent anti-TNF-treatments, on the contrary, result in brain toxoplasmosis in RA patients [12, 18]. Furthermore, the stimulation of certain toll-like receptors and the immune responsiveness in toxoplasmosis increases the production of autoimmune immunoglobulins [19].

Various studies in different parts of the world have found a link between *Toxoplasma* antibodies seropositivity and RA [20]. Chronic disease patients as those with RA have a higher occurrence of toxoplasmosis than normal people, that may be clarified as the usage of immunosuppressive medications to manage chronic disorders makes patients more susceptible to illnesses, including toxoplasmosis [21].

Toxoplasma gondii has also been demonstrated to increase interleukin 17 (IL-17) expression, which is essential, as this interleukin is linked to the pathogenesis of several autoimmune illnesses, RA is one among them. All of these variables can accelerate the course of chronic illnesses and induce acute toxoplasmosis, threatening the patient's life [22, 23].

Patients suffering from RA were at risk for toxoplasmosis, as a result, preventative and screening programs, as well as toxoplasmosis therapy, should be prioritized in individuals with RA to avoid severe complications of this parasitic infection in rheumatoid patients who were taking immunosuppressive therapy [14]. Also, this study found that RA was more

prevalent in females than in men, which was consistent with other studies [13, 24], and that a significant difference was identified by gender (P=0.0001).

Additionally, the current study found that seropositive toxoplasma Abs occur more frequently in patients with age group between (31-49) years with the uppermost ratio of *T. gondii* antibodies (43.7%) than in the other age groups, potentially due to more frequent interaction with cats and kitchen activities in this age group, and this finding was similar to other studies as a study by Dreaj et al.[13]. Thus, investigation of this age group for toxoplasma antibodies is highly recommended to detect the disease early and avoid further complications. This study does not identify any correlation between seropositivity and the type of biological drug administered.

Limitation of this study is the relatively small sample size which used, further studies with larger samples are required to confirm the results and study the prevalence of toxoplasma antibodies in rheumatoid arthritis.

CONCLUSION

There is an association between long-term biological treatments for RA patients and the onset of toxoplasmosis, which underscores the value of conducting serological tests for early detection in autoimmune patients to prevent serious complications.

ETHICAL DECLARATIONS

- **Ethics Approval and Consent to Participate**

  Ethical permission was obtained from the College of Medicine - University of Mosul (reference number: UOM/COM/MREC/22-23(14) dated 29/12/2022) and informed consent was secured from all participants.

- **Consent for Publication**

  Non.

- **Availability of Data and Material**

  The datasets are available from the corresponding author upon reasonable request.

- **Competing Interests**

  The authors declare that there is no conflict of interest.

- **Funding**

  Self funded.

- **Authors' Contributions**

  All authors contributed significantly, directly, and intellectually to the work and consented to its publication.

REFERENCES

[1] Jahid M, Khan KU, Ahmed RS, et al. Overview of rheumatoid arthritis and scientific understanding of the disease. Mediterranean Journal of Rheumatology 2023;34(3):284–291. https://doi.org/10.31138/mjr.20230801.oo.

[2] Deshmukh R. Rheumatoid arthritis: Pathophysiology, current therapeutic strategies and recent advances in targeted drug delivery system. Materials Today Communications 2023;35:105877. https://doi.org/10.1016/j.mtcomm.2023.105877.

[3] Law ST, Taylor PC. Role of biological agents in treatment of rheumatoid arthritis. Pharmacological research 2019;150:104497. https://doi.org/10.1016/j.phrs.2019.104497.

[4] Mohammed AM, Zayni SM, AL-Anee MM, Corial FI, Al-Rubaee A. Diagnostic and predictive values of IL-6 in a group of Iraqi patients with rheumatoid arthritis. Journal of the Faculty of Medicine Baghdad 2023;65(2):116–121. https://doi.org/10.32007/jfacmedbagdad.2044.

[5] Ghobadinezhad F, Ebrahimi N, Mozaffari F, Moradi N, Beiranvand S, Pournazari M, et al. The emerging role of regulatory cell-based therapy in autoimmune disease. Frontiers in Immunology 2022;13:1075813. https://doi.org/10.3389/fimmu.2022.1075813.

[6] Omran RH, Zahra'a AA, Alrawi AA. Evaluation of Some New Cytokines in Rheumatoid Arthritis. Journal of the Faculty of Medicine Baghdad 2022;64(3):159–162. https://doi.org/10.32007/jfacmedbagdad.6431963.

[7] Lahiri M, Dixon WG. Risk of infection with biologic antirheumatic therapies in patients with rheumatoid arthritis. Best practice & research Clinical rheumatology 2015;29(2):290–305. https://doi.org/10.1016/j.berh.2015.05.009.

[8] Alkubaisi SAMS, Al-Zubaidy IAHS. Toxoplasmosis in females from Al-Anbar, Iraq. Journal of the Faculty of

Medicine Baghdad 2023;65(1):74–78. https://doi.org/10.32007/jfacmedbagdad.6512064.

[9] Sanchez SG, Besteiro S. The pathogenicity and virulence of Toxoplasma gondii. Virulence 2021;12(1):3095–3114. https://doi.org/10.1080/21505594.2021.2012346.

[10] Greigert V, Bittich-Fahmi F, Pfaff AW. Pathophysiology of ocular toxoplasmosis: Facts and open questions. PLOS Neglected Tropical Diseases 2020;14(12):e0008905. https://doi.org/10.1371/journal.pntd.0008905.

[11] Tian AL, Gu YL, Zhou N, Cong W, Li GX, Elsheikha HM, et al. Seroprevalence of Toxoplasma gondii infection in arthritis patients in eastern China. Infectious diseases of poverty 2017;6:1–7. https://doi.org/10.1186/s40249-017-0367-2.

[12] Hosseininejad Z, Sharif M, Sarvi S, Amouei A, Hosseini SA, Nayeri Chegeni T, et al. Toxoplasmosis seroprevalence in rheumatoid arthritis patients: a systematic review and meta-analysis. PLoS neglected tropical diseases 2018;12(6):e0006545. https://doi.org/10.1371/journal.pntd.0006545.

[13] Dreaj HA, Hathal HD, Abbas AAH. Seropositivity of Toxoplasma gondii antibodies in rheumatoid arthritis patients under treatment with TNF-? antagonists. Biomedicine 2022;42(2):314–317. https://doi.org/10.51248/.v42i2.934.

[14] Zarean M, Mastroeni P, Moghaddas E, Farash BRH, Raouf-Rahmati A, Jamali J, et al. Toxoplasmosis frequency rate in rheumatoid arthritis patients in Northeastern Iran. Iranian Journal of Parasitology 2022;17(3):325. https://doi.org/10.18502/ijpa.v17i3.10622.

[15] Vargas-Villavicencio JA, Cañedo-Solares I, Correa D. Anti-toxoplasma gondii IgM long persistence: what are the underlying mechanisms? Microorganisms 2022;10(8):1659. https://doi.org/10.3390/microorganisms10081659.

[16] Lewis JM, Clifford S, Nsutebu E, Toxoplasmosis in immunosuppressed patients. Oxford University Press; 2015. https://doi.org/10.1093/rheumatology/kev115.

[17] Sana M, Rashid M, Rashid I, Akbar H, Gomez-Marin JE, Dimier-Poisson I. Immune response against toxoplasmosis—some recent updates RH: Toxoplasma gondii immune response. International journal of immunopathology and pharmacology 2022;36:03946320221078436. https://doi.org/10.1177/03946320221078436.

[18] Al-Derzi AR. The Contribution of Serum Anti–cyclic Citrullinated Peptide Antibody and Matrix Metalloproteinase-3 in Predicting the Activity of Rheumatoid Arthritis Disease. Journal of the Faculty of Medicine Baghdad 2017;59(2):138–144. https://doi.org/10.32007/jfacmedbagdad.592124.

[19] Sasai M, Yamamoto M. Innate, adaptive, and cell-autonomous immunity against Toxoplasma gondii infection. Experimental & molecular medicine 2019;51(12):1–10. https://doi.org/10.1038/s12276-019-0353-9.

[20] El-Henawy AA, Hafez EAR, Nabih N, Shalaby NM, Mashaly M. Anti-Toxoplasma antibodies in Egyptian rheumatoid arthritis patients. Rheumatology international 2017;37:785–790. https://doi.org/10.1007/s00296-017-3703-8.

[21] Wang ZD, Liu HH, Ma ZX, Ma HY, Li ZY, Yang ZB, et al. Toxoplasma gondii infection in immunocompromised patients: a systematic review and meta-analysis. Frontiers in microbiology 2017;8:389. https://doi.org/10.3389/fmicb.2017.00389.

[22] Washino T, Moroda M, Iwakura Y, Aosai F. Toxoplasma gondii infection inhibits Th17-mediated spontaneous development of arthritis in interleukin-1 receptor antagonist-deficient mice. Infection and immunity 2012;80(4):1437–1444. https://doi.org/10.1128/iai.05680-11.

[23] McInnes IB, Gravallese EM. Immune-mediated inflammatory disease therapeutics: past, present and future. Nature Reviews Immunology 2021;21(10):680–686. https://doi.org/10.1038/s41577-021-00603-1.

[24] El-Sayed NM, Kishik SM, Fawzy RM. The current status of Toxoplasma gondii infection among Egyptian rheumatoid arthritis patients. Asian Pacific Journal of Tropical Disease 2016;6(10):797–801. https://doi.org/10.1016/S2222-1808(16)61133-7.