ISSN: 1607-8322, e-ISSN: 2220-5799 Anaesthesia, Pain & Intensive Care
Vol 28(4); August 2024 DOI: 10.35975/apic.v28i4.2510

ORIGINAL RESEARCH PAIN MANAGEMENT

A case-control study of the association of toxoplasmosis with antirheumatic therapy in rheumatoid arthritis

Rabie G. Abdullah [1], Souzan H. Eassa [2], Fouad K. Mohammad [3]

Author affiliations:

1. Rabie G. Abdullah, Department of Pharmacology, College of Pharmacy, University of Duhok, Duhok, Kurdistan Region, Iraq; E-mail: rabie.gabriel@uod.ac
2. Souzan H. Eassa, Department of Basic Sciences, College of Medicine, University of Duhok, Duhok, Kurdistan Region, Iraq; Molecular and Microbiology Division, School of Medicine, University of Kurdistan Hewlêr, Erbil, Iraq; E-mail: souzan.hussain@ukh.edu.krd
3. Fouad K. Mohammad, Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq; College of Nursing, American University of Kurdistan, Duhok, Kurdistan Region, Iraq; E-mail: fouadmohammad@yahoo.com

Correspondence: Fouad K. Mohammad, E-mail: fouadmohammad@yahoo.com

ABSTRACT

Background & Objective: Toxoplasmosis is a globally prevalent opportunistic zoonotic infection. Rheumatoid arthritis (RA) is an autoimmune disease which is manifested as joint inflammation, pain, stiffness, and swelling. The patients may contract toxoplasmosis, especially when receiving antirheumatic immunosuppressive therapy. However conflicting results have been reported on toxoplasmosis in RA patients. We aimed to evaluate *Toxoplasma gondii* positivity and associated risk factors in patients with RA in Duhok province, Iraq, and to assess the role of antirheumatic therapy in toxoplasmosis and the possibility of its reactivation.

Methodology: This case-control study was carried out in Duhok, Kurdistan Region, Iraq, from February 2022 to June 2023. A total of 88 RA patients, with and without concurrent antirheumatic therapy, were recruited. The diagnosis of RA was according to the 2010 American College of Rheumatology/European League. RA-patients were categorized into three groups: Group 1 - no-therapy (n = 14); Group 2 - conventional therapy (n = 49) and Group 3 - biologics in combination with conventional medications (n = 25). Healthy subjects (n = 61) were also selected as controls. Plasma samples obtained from the participants were screened for *T. gondii* infection using Human anti-*Toxoplasma gondii* antibody IgG. IgG positive samples were tested utilizing human anti-*Toxoplasma gondii* antibody IgM ELISA Kit and the avidity *T. gondii* IgG ELISA kit was used to determine the state of infection.

Results: Of the 88 RA patients, with or without antirheumatic therapy, 76 (86.36%) were females and 12 (13.64%) were males. Patients on therapy (n = 74) received several conventional antirheumatic drugs of which methotrexate was the most frequently used one (86.36%). Among biological therapies, etanercept, a TNF-alpha inhibitor, was mostly used (13.51%).

The toxoplasmosis was significantly less in the older age group (> 50 y) compared to individuals under 30 y. Gender had no significant effect. *Toxoplasma* IgG positivity was found to be significantly higher in the three groups of RA patients in comparison with healthy controls. Further assessment of anti-*Toxoplasma* IgM positivity and *Toxoplasma* IgG avidity results to identify the state of *T. gondii* infection in anti-*Toxoplasma* IgG positive subjects revealed that the frequency of reactivation of toxoplasmosis in RA patient groups was not significantly different from that of the control counterparts.

Conclusion: The study showed higher seroprevalence of anti-*Toxoplasma* antibodies in patients with RA irrespective of therapy; therefore, we could consider toxoplasmosis interaction with RA in the pathogenesis of both diseases. This seroprevalence was higher in younger age groups, but with no significant role of gender, duration of disease, duration of therapy and its type. No significant difference was noted in rates of reactivation of latent *T. gondii* infection among RA patients and healthy controls.

Abbreviations: CI - Confidence interval; O - Odds ratio; RA - Rheumatoid arthritis; SD - Standard deviation; *T. gondii* - *Toxoplasma gondii*; TNF - Tumor necrosis factor.

Keywords: Autoimmune disease; Rheumatoid arthritis; Antirheumatic drugs; *Toxoplasma gondii*; *Toxoplasma* seroprevalence; *Toxoplasma* IgG avidity.

Citation: Abdullah RG, Eassa SH, Mohammad FK**.** A case-control study of the association of toxoplasmosis with antirheumatic therapy in rheumatoid arthritis. Anaesth, pain intensive care 2024;28(4):674−680; **DOI:** 10.35975/apic.v28i4.2510

Received: May 14, 2024; **Reviewed:** June 28, 2024; **Accepted:** June 28, 2024

1. INTRODUCTION

Toxoplasmosis is a globally prevalent, opportunistic protozoan, zoonotic infection. It is caused by the obligate intracellular protozoan parasite known as *Toxoplasma gondii* (*T. gondii*).[1] All warm-blooded animals are susceptible to infection by the parasite that causes chronic infection in about one-third of the human population.[1,2] Human infection usually occurs through contact with contaminated cat feces or consuming inadequately cooked meat derived from an infected animal.[1,2]

Rheumatoid arthritis (RA) is an autoimmune disease, which is manifested as joint inflammation, resulting in signs and symptoms that include pain, stiffness, swelling, and reduced functionality, with substantial morbidity and diminished quality of life of the affected individuals.[3] The age-standardized global prevalence rate of RA was estimated to be 208.8 cases per 100,000 population, with a higher preponderance in females (82.76%) compared to males (17.24%).[4] In Iraq, the prevalence was estimated to be 104.5 per 100,000 population,[4] with higher rates in females compared to males.[5,6] The latter study was a preliminary one we conduced to examine plasma cholinesterase activity in RA patients with toxoplasmosis.[6]

The primary focus of dealing with RA, in addition to an early diagnosis, is to effectively manage pain and inflammatory responses, improve mobility, and minimize the risk of joint degeneration and deformity.[3,7] Major therapeutic options against RA include conventional antirheumatic drugs and biologic agents.[3,7,8] However, the administration of immunosuppressive medications, including both conventional and biologic therapies, has been associated with an elevated vulnerability to opportunistic infections like tuberculosis, histoplasmosis, and toxoplasmosis.[9,10] Within this context, the association between toxoplasmosis and RA is complex and largely unclear from the pathophysiological aspects of the disease.[10,11]

Severe forms of toxoplasmosis may occur in RA patients who receive immunosuppressive therapy, especially the anti-tumor necrosis factor (anti-TNF) medications.[12,13] However, in spite of reports of toxoplasmosis and/or RA in Iraqi patients a few studies addressed the role of antirheumatic immunosuppressive therapy, though with conflicting results,[5,6,14-16] regarding the possible association of toxoplasmosis with RA.[11] This research aimed to further expand our previous study[6] and evaluate *T. gondii* positivity and associated risk factors in patients with RA in Duhok province, Iraq, and to assess the role of antirheumatic therapy in toxoplasmosis and the possibility of its reactivation.

2. METHODOLOGY

This case-control study was carried out in Duhok, Kurdistan Region, Iraq, from February 2022 to June 2023. The aims and significance of the study were explained to all participants and signed informed consents were obtained from them. The study received approval from the Committee of Higher Studies, College of Pharmacy, University of Duhok, Iraq (No. 320, 4/8/2021) and the final approval from the Research Ethics Committee, Duhok Directorate General of Health, and registered with the reference number 15092021-9-14 R1, August 7, 2023

2.1. Inclusion and exclusion criteria

We recruited 88 RA patients, with and without concurrent antirheumatic therapy, from the Duhok Center of Rheumatic Diseases & Medical Rehabilitation, Duhok, Iraq. The patients had been diagnosed by rheumatologists based on the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria.[17] Patients with moderate to severe comorbidities were excluded from the study. Pregnant women and patients with past surgical procedures on the joints were also excluded from the study.

2.2. Patients groups

RA patients were categorized into three groups: RA patients who did not receive therapy up to the time of the interview (RA Group 1, n = 14), and those who received conventional therapy including methotrexate, corticosteroids, and other disease-modifying antirheumatic drugs (RA Group 2, n = 49), and the last group comprised patients who were given biologics (such as infliximab, adalimumab, etanercept) mostly in combination with conventional medications (RA Group 3, n = 25). Healthy subjects (n = 61) were selected as

controls from the staff of the rheumatic center, and from blood donors who attended Duhok blood bank.

2.3. Blood sampling / *T. gondii* seroprevalence assays

About 5 mL of venous blood samples were obtained from the patients and controls and they were collected into heparinized vacutainer tubes. Plasma was separated by centrifuging and stored at –20°C pending analysis within one month. All samples were screened for *T. gondii* infection using Human anti-*Toxoplasma gondii* antibody IgG ELISA Kit (Cat. No ED0537Hu, BT LAB Co., China). IgG positive samples were tested utilizing Human anti-*Toxoplasma gondii* antibody IgM ELISA Kit (Cat. No ED0538Hu, BT LAB Co., China) and the avidity *T. gondii* IgG ELISA kit (NovaLisa®, Dietzenbach, Germany) was used to determine the state of infection (whether the infection was current, past, or a reactivation one) according to the manufacturer's instructions.

2.4. Statistical analysis

Continuous data are shown as mean and standard deviation (SD), and categorical data are presented as numbers and percentages. The Odds ratio (OR) with 95% confidence interval (CI) were estimated by binary logistic regression with backward selection of variables at $P < 0.2$.[18] Fisher's exact probability test was performed to compare the state of infection among groups. The statistical package Minitab software (Version 21.4.1, Minitab Inc., PA, USA), as well as the statistical software PAST4.13[19] were used for statistical analyses. A $P < 0.05$ was considered statistically significant.

3. RESULTS

This study included 88 RA patients with or without antirheumatic therapy. Seventy-six (86.36%) patients were females and 12 (13.64%) were males. Their mean age was 44.51 ± 13.404 y, and their mean body mass index (BMI) was 30.463 ± 7.554 kg/m^2 (Table 1).

Table 1: Demography of rheumatoid arthritis (RA) patients and healthy controls

Variables	RA patients (n = 88)	Healthy controls (n = 61)	p-value
Gender			
• Female	76 (86.36)	22 (36.07)	0.0001
• Male	12 (13.64)	39 (63.93)	
Age (y)	44.51 ± 13.404	37.21 ± 9.91	0.0001
BMI (kg/m^2)	30.463 ± 7.554	27.88 ± 4.538	0.015

Data presented as mean ± SD or n (%)

Table 2: Medications used by 74 rheumatoid arthritis (RA) patients. [n (%)]

Medications	RA patients received therapy
A. Conventional agents	
• Methotrexate	56 (75.68)
• Prednisolone	8 (10.81)
• Hydroxychloroquine	3 (4.05)
• Azathioprine	1 (1.35)
• Deflazacort	1 (1.35)
• Sulfasalazine	1 (1.35)
B. Biological agents	
• Etanercept	10 (13.51)
• Infliximab	6 (8.11)
• Tocilizumab	5 (6.76)
• Adalimumab	2 (2.70)
• Rituximab	2 (2.70)

Patients on antirheumatic therapy (n = 74) received several conventional antirheumatic drugs of which methotrexate was the most frequently utilized one (n = 56, 86.36%). Among biological therapies, etanercept, a TNF-alpha inhibitor, was mostly used (n = 10, 13.51%) (Table 2).

The frequency of occurrence of toxoplasmosis was significantly lesser in the older age group (> 50 y) when compared to individuals under 30 y of age (OR, 0.11; 95% CI, 0.03–0.49; P = 0.004) (Table 3). Within this context, gender had no statistically significant effect.

Toxoplasma IgG positivity was found to be significantly higher in RA patients (n = 14) without therapy (OR, 14.01; 95% CI, 2.82–69.55; P = 0.001), RA patients (n = 49) who received conventional therapy (OR, 5.71; 95% CI, 1.67–19.52; P = 0.006), and those (n = 25) with combination biologic and conventional therapy (OR, 3.90; 95% CI, 1.10–13.86; P = 0.035) in comparison with healthy controls (Table 3).

Upon further assessment of anti-*Toxoplasma* IgM seroprevalence and *Toxoplasma* IgG avidity results, we were able to identify the state of *T. gondii* infection in anti-*Toxoplasma* IgG-positive subjects (Table 4). As total numbers of *T. gondii*-infected patients varied from 8 to 17 among the tested groups, current and past infections also varied accordingly from 0 to 3 and 3 to 8,

Table 3: Association of toxoplasmosis with demographics in rheumatoid arthritis (RA) patients (n = 88), and non-RA healthy controls (n = 61)

Variables	Frequency	*Toxoplasma* IgG positive n (%)	*Toxoplasma* IgG negative n (%)	Odds ratio (95% C.I.)	P-value
Age group					
< 30 y	29	15 (51.7)	14 (48.3)	1.00 (reference)	-
30-50 y	85	27 (31.8)	58 (68.2)	0.37 (0.13, 1.12)	0.078
> 50 y	35	9 (25.7)	26 (74.3)	0.11 (0.03, 0.49)	0.004
Gender					
Female	98	37 (37.8)	61 (62.2)	1.00 (reference)	-
Male	51	14 (27.5)	37 (72.5)	0.62 (0.20, 1.90)	0.406
Participants					
Control	61	16 (26.2)	45 (73.8)	1.00 (reference)	-
RA group 1	14	8 (57.1)	6 (42.9)	14.01 (2.82, 69.55)	0.001
RA group 2	49	17 (34.7)	32 (65.3)	5.71 (1.67, 19.52)	0.006
RA group 3	25	10 (40.0)	15 (60.0)	3.90 (1.10, 13.86)	0.035

RA Group 1 = RA patients without therapy, RA Group 2 = RA patients received conventional therapy, RA Group 3 = RA patients received combination therapy (biologic agent + conventional drugs)

Table 4: State of *T gondii* infection in anti-*Toxoplasma* IgG positive subjects

Groups	Current infection n (%)	Past infection n (%)	Reactivation n (%)	Undefined n (%)	Total
Control	2 (12.50)	5 (31.25)	5 (31.25)	4 (25.00)	16
RA Group 1	3 (37.50)	3 (37.50)	0 (0.00)	2 (25.00)	8
RA Group 2	3 (17.65)	8 (47.06)	3 (17.65)	3 (17.65)	17
RA Group 3	0 (0.00)	5 (50.00)	2 (20.00)	3 (30.00)	10

No statistically significant differences among groups.

RA Group 1 = RA patients without therapy, RA Group 2 = RA patients received conventional therapy, RA Group 3 = RA patients received combination therapy (biologic agent + conventional drugs)

respectively (Table 4). Most importantly and within the context of the state of infection, the results revealed that the frequency of reactivation of toxoplasmosis in RA patient groups was not significantly different among themselves and from that of the control counterparts. To this end, as shown in Table 4, the percentages of reactivation, which were of low values, ranged from 0% to only 3% in RA patients when compared to that of the control group (5%).

4. DISCUSSION

The present study aimed to assess the seroprevalence of *T. gondii* infection among individuals diagnosed with RA in Duhok, Iraq. The high percentage of RA in female Iraqi patients from Duhok was in agreement with those reported locally in Iraq or globally.[4-6,15] However, other studies did not report gender effects on the occurrence of RA.[11,16,20] The types of antirheumatic therapy recorded in the present study are in accordance with recent RA findings in Duhok, however, with varying adherence rates to medications.[15] The main reason to start antirheumatic treatments is to stop the progress of RA and improve the quality of life of the patients.[3-5,7,8]

A unique finding of the present study showed that the rates of toxoplasmosis in patients with RA who received no treatment, conventional therapy were considerably higher than that of the controls (26.2%). To this end, gender seemed not to significantly affect this frequency in RA patients. Besides, regarding toxoplasmosis frequency, no statistically significant differences were found among the subgroups of RA patients, and there was no evidence of association between the RA duration,

or the duration of therapy with the seroprevalence of anti-*T. gondii* IgG (data not shown in the results). Therefore, the impact of duration of RA therapy on the occurrence of toxoplasmosis needs additional in-depth exploration.

Our results are in accordance with those of Kuba et al.[16] who demonstrated significantly high prevalence of toxoplasmosis among Iraqi RA patients receiving methotrexate (33.3%) and RA group without treatment (36%) when compared to control group (12%). Similarly, earlier studies from Egypt and Turkey, found a statistically greater frequency of toxoplasmosis in patients with RA versus healthy controls, without significant dissimilarity among patients receiving conventional antirheumatic medications and those treated with a biologic agent.[9,21]

Several studies conducted on RA patients also concluded greater frequency of *T. gondii* seropositivity in comparison to healthy participants without referring to therapy received or its impact on toxoplasmosis.[11,22,23] In addition, others assessed the influence of RA duration or the age of the patients on the prevalence of toxoplasmosis without significant outcome.[11,20,24]

While some studies revealed no significant association of age with the prevalence of toxoplasmosis among RA patients;[11,23,25] in contrast we report significantly lesser frequency in the older age group (> 50 y). Our results are in agreement to those of others.[26,27] However, the latter age effect on the seropositivity of toxoplasmosis has been reported earlier without any correlation with the pathogenesis of RA.[24] The present younger age-related finding of toxoplasmosis could be attributed to increased frequency of cat ownership (the primary source of toxoplasmosis[1,2]) in young individuals in our local community.[28] Nevertheless, autoimmunity status of RA patients, being immunocompromised, might be a factor in the prevalence of toxoplasmosis among them.[10,12,13]

Various studies have postulated various hypotheses to explain the high prevalence of toxoplasmosis in patients with chronic autoimmunity conditions like RA. The protozoan *T. gondii* may trigger a strong and long-lasting cellular response mediated by T helper cells, a response which is characterized by the production of many pro-inflammatory cytokines that help in defending the host's cells against the parasite, but at the same time, induce significant pathological and immunological alterations, ultimately leading to an autoimmune condition.[1,10,29] It is also possible that immunosuppressive therapy for RA, such as TNF-alpha antagonists, cause dormant toxoplasmosis to be reactivated in patients and increase their susceptibility to opportunistic infections.[25] *T. gondii* protozoon upregulates the expression of interleukin-17 in infected people; this cytokine has a role in the development of several autoimmune disorders, hence establishing a notable connection between toxoplasmosis and autoimmune illnesses.[30] The increased vulnerability of individuals with RA toward opportunistic infections may also be elucidated by the pathobiology of the illness itself. The premature degeneration of the immune system in immunological-mediated disorders like RA may contribute to the impairment of crucial immune processes, resulting in reduced protection against infectious parasites.[30]

The present study employed human anti-*T. gondii* antibody IgM ELISA test and avidity *T. gondii* IgG ELISA test in *Toxoplasma* IgG positive samples to identify the state of infection, particularly, the reactivation one. The results showed non-significant difference in the rate of *Toxoplasma* reactivation among RA groups themselves and when they were compared to healthy controls. This notion was supported by low percentages of reactivation, which were 0% to 3% in RA patients versus the 5% of the control group. A similar study also reported no significant differences in *Toxoplasma* seropositivity among RA treatment and non-treatment groups.[16]

Nevertheless, the presence of anti-*Toxoplasma* IgM antibodies in serum of *T. gondii* IgG positive individuals may indicate a recent infection, but not necessarily an activation of latent toxoplasmosis. The application of *T. gondii* IgG avidity test, as we did in the present study, is a crucial attempt to differentiate recent toxoplasmosis from reactivation of latent infection.[14,31] However, limited number of patients in each RA group in the present study, may have contributed to this non-significant reactivation outcome. Furthermore, we could not delineate false negative expression of anti-*Toxoplasma* IgG among the RA groups, since it was reported that such a phenomenon can find its way in patients, especially the elderly, with dysregulation of immunity.[11-13,32,33]

5. LIMITATIONS

In the present study, the sample size of RA patients without therapy was small (n = 14). However, this is anticipated due to the need for intense and prompt therapeutic action as existing joint erosions cannot be reversed, given that the welfare of the patients is contingent upon the effectiveness of treatment, along with other variables that impact the course of the disease[6-7]

This may explain the lack of such a group (RA patients with no therapy) in the majority of similar studies mentioned previously with few exceptions.[16,20] False negative results for anti-*Toxoplasma* IgG can also occur, though uncommon, in people with

hypogammaglobulinemia or severe immunosuppression, especially in elderly.

Another obscure point that needs additional exploration is the relationship of toxoplasmosis to the duration of therapy in RA patients.

6. CONCLUSION

Our study showed higher seroprevalence of anti-Toxoplasma antibodies in patients with rheumatoid arthritis irrespective of therapy when compared to healthy individuals. Therefore, we should consider toxoplasmosis interaction with rheumatoid arthritis in the pathogenesis of both diseases.

This seropositivity was noted to be higher in younger age groups, but with no significant role of gender, duration of disease, duration of therapy and its type. No significant difference was noted in rates of reactivation of latent *T. gondii* infection among rheumatoid arthritis patients and healthy controls.

Additional research should be conducted to elucidate the significance of *T. gondii* infection in patients with rheumatoid arthritis and its relevance to antirheumatic therapy as well as the duration of such therapy. Despites these findings, the risk of reactivation of latent toxoplasmosis should not be ruled out, especially in patients receiving immunosuppressive medications. Hence, extra caution should be taken when prescribing these therapeutic agents in toxoplasma positive rheumatoid arthritis patients.

7. Data availability

The numerical data of this study is available with the first author.

8. Conflict of interests

The authors declare no conflicts of interest with respect to the authorship and/or publication of this article.

9. Acknowledgments

This report represents a portion of a dissertation to be submitted by the first author to the University of Duhok, Iraq in partial fulfillment of the requirements for the Ph.D. degree in Clinical Pharmacology. The authors thank the College of Pharmacy, University of Duhok for providing facilities and supplies to conduct this study.

10. Authors' contribution

RGA: Dealt with the participants, obtained blood samples and executed laboratory assays; conducted literature search, statistical analyses and shared in drafting the manuscript.

SHE & FKM: Conceptualized and supervised the study, shared in literature search, statistical analyses and drafting the manuscript. All authors have read the manuscript and approve it for publication.

11. REFERENCES

1. Dubey JP. Toxoplasmosis of Animals and Humans, 3rd ed, CRC Press, Boca Raton, FL, USA; 2022.
2. Dámek F, Swart A, Waap H, Jokelainen P, Le Roux D, Deksne G, et al. Systematic review and modelling of age-dependent prevalence of Toxoplasma gondii in livestock, wildlife and felids in Europe. Pathogens. 2023;12(1):97. [PubMed] DOI: 10.3390/pathogens12010097
3. Gravallese EM, Firestein GS. Rheumatoid arthritis - common origins, divergent mechanisms. N Engl J Med. 2023;388(6):529-42. [PubMed] DOI: 10.1056/NEJMra2103726
4. GBD 2021 Rheumatoid Arthritis Collaborators. Global, regional, and national burden of rheumatoid arthritis, 1990-2020, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. Lancet Rheumatol. 2023;5(10). [PubMed] DOI: 10.1016/S2665-9913(23)00211-4
5. Abbas KS, Hasan AS, Tareq NL. Assessment of the impact of different rheumatoid arthritis stages on the quality of life of a sample of Iraqi patients. J Adv Pharm Educ Res. 2023;13(1):122-6. DOI: 10.51847/41Nl6iG253
6. Abdullah RG, Eassa SH, Mohammad FK. Plasma cholinesterase activity in patients with rheumatoid arthritis and toxoplasmosis. Cureus 2023;15(12). [PubMed] DOI: 10.7759/cureus.50979
7. Mueller AL, Payandeh Z, Mohammadkhani N, Mubarak SMH, Zakeri A, Alagheband Bahrami A, et al. Recent advances in understanding the pathogenesis of rheumatoid arthritis: new treatment strategies. Cells. 2021;10(11):3017. [PubMed] DOI: 10.3390/cells10113017
8. Singh JA. Treatment guidelines in rheumatoid arthritis. Rheum Dis Clin North Am. 2022;48(3):679-89. [PubMed] DOI: 10.1016/j.rdc.2022.03.005
9. El-Henawy AA, Hafez EAR, Nabih N, Shalaby NM, Mashaly M. Anti-toxoplasma antibodies in Egyptian rheumatoid arthritis patients. Rheumatol Int. 2017;37(5):785-90. [PubMed] DOI: 10.1007/s00296-017-3703-8
10. 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 Negl Trop Dis. 2018;12(6). [PubMed] DOI: 10.1371/journal.pntd.0006545

11. Dong W, Zhong Q, Gu YL, Liang N, Zhou YH, Cong XM, et al. Is Toxoplasma gondii infection a concern in individuals with rheumatic diseases? Evidence from a case-control study based on serological diagnosis. Microb Pathog. 2023;182:106257. [PubMed] DOI: 10.1016/j.micpath.2023.106257

12. Hill B, Wyatt N, Ennis D. Cerebral Toxoplasmosis in a rheumatoid arthritis patient on immunosuppressive therapy. Cureus. 2020;12(6). [PubMed] DOI: 10.7759/cureus.8547

13. Durieux MF, Lopez JG, Banjari M, Passebosc-Faure K, Brenier-Pinchart MP, Paris L, et al. Toxoplasmosis in patients with an autoimmune disease and immunosuppressive agents: a multicenter study and literature review. PLoS Negl Trop Dis. 2022;16(8). [PubMed] DOI: 10.1371/journal.pntd.0010691

14. Salih JM, Mero WMS, Eassa SH. Seroprevalence and some demographic factors associated with Toxoplasma gondii infection among male population in Duhok Province/Iraq. Baghdad Sci J. 2020;17:431-6. DOI: 10.21123/bsj.2020.17.2.0431

15. Kasim HF, Salih HM, Eassa SH. Adherence to biologic drugs among patients with immune mediated inflammatory diseases in Duhok Governorate. Duhok Med J. 2020;14(1):63-6. [FreeFullText]

16. Kuba RH, Zghair KH, Alosami MH. Detection of toxoplasma antibodies and TNF-α in rheumatoid arthritis patients treated with methotrexate. Iraqi J Sci. 2023;55(4A):1535-40. [FreeFullText]

17. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2010;69(9):1580-8. [PubMed] DOI: 10.1136/ard.2010.138461

18. Antwi E, Groenwold RH, Browne JL, Franx A, Agyepong IA, Koram KA, et al. Development and validation of a prediction model for gestational hypertension in a Ghanaian cohort. BMJ Open. 2017;7(1). [PubMed] DOI: 10.1136/bmjopen-2016-012670

19. Hammer Ø, Harper DAT, Ryan PD. PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron. 2001;4(1):1. [FreeFullText]

20. El-Sayed NM, Kishik SG, Fawzy RM. The current status of Toxoplasma gondii infection among Egyptian rheumatoid arthritis patients. Asian Pac J Trop Med. 2016;6:797–801.

21. İnal A, Taş D. Toxoplasma gondii seroprevalence in rheumatoid arthritis patients treated with biological agents. J Surg Med. 2019;3(3):239-41. DOI: 10.28982/josam.523350

22. Al-Oqaily MA, Al-Ubaidi IK. Prevalence of toxoplasmosis in Iraqi rheumatoid arthritis patients and detection levels of MCP-1 and TGF-β chemokines during infection. Int J Sci Nature. 2017;8(4):824-9. [FreeFullText]

23. Dreaj HA, Hathal HD, Hassan Abbas AA. Seropositivity of Toxoplasma gondii antibodies in rheumatoid arthritis patients under treatment with TNF-α Antagonists. Biomedicine. 2022;42(2):314-7. [FreeFullText]

24. Fischer S, Agmon-Levin N, Shapira Y, Porat Katz BS, Graell E, Cervera R, et al. Toxoplasma gondii: bystander or cofactor in rheumatoid arthritis. Immunol Res. 2013 Jul;56(2-3):287-92. [PubMed] DOI: 10.1007/s12026-013-8402-2

25. Massoori L, Molazadeh M, Rezaei N, Alizadeh S, Hassanpour H, Badirzadeh A. Development of rheumatoid arthritis by toxoplasmosis in Iranian patients. J Kerman Univ Med Sci. 2021;28(4):412-419. DOI: 10.22062/jkmu.2021.91724

26. Tian A-L, Gu YL, Zhou N, Cong W, Li GX, Elsheikha HM, et al. Seroprevalence of Toxoplasma gondii infection in arthritis patients in eastern China. Infec Dis of Pov. 2017;6(1):153. [PubMed] DOI: 10.1186/s40249-017-0367-2

27. Abdul Ameer Jaber K, Aamer Noori R. Comparisons of Toxoplasma gondii prevalence in rural and urban areas by serological methods in Al-Najaf province, Iraq. Arch Razi Inst 2021;76(6):1695-701. [PubMed] DOI: 10.22092/ari.2021.356315.1822

28. Tamimi NS, Al-Lami SAD, Abbas SF. Validation of a questionnaire for assessment of Iraqi domestic cat's behavior. Ann Roman Soc Cell Biol. 2023;27(1):282-8. [PubMed]

29. Yarovinsky F. Innate immunity to Toxoplasma gondii infection. Nature Rev Immunol. 2014;14(2):109-21. [PubMed] DOI: 10.1038/nri3598

30. Listing J, Gerhold K, Zink A. The risk of infections associated with rheumatoid arthritis, with its comorbidity and treatment. Rheumatology (Oxford). 2013 Jan;52(1):53-61. [PubMed] DOI: 10.1093/rheumatology/kes305

31. Teimouri A, Mohtasebi S, Kazemirad E, Keshavarz H. Role of Toxoplasma gondii IgG avidity testing in discriminating between acute and chronic toxoplasmosis in pregnancy. J Clin Microbiol. 2020 Aug 24;58(9). [PubMed] DOI: 10.1128/JCM.00505-20

32. Rajput R, Denniston AK, Murray PI. False negative toxoplasma serology in an immunocompromised patient with PCR positive ocular toxoplasmosis. Ocul Immunol Inflamm. 2018;26(8):1200-2. [PubMed] DOI: 10.1080/09273948.2017.1332769

33. Jean-Pierre V, Miozzo J, Fricker-Hidalgo H, Garnaud C, Robert MG, Pelloux H, et al. Serological diagnosis of toxoplasmosis: evaluation of the commercial test recomLine Toxoplasma IgG immunoblot (Mikrogen) based on recombinant antigens. Parasite. 2022;29:52. [PubMed] DOI: 10.1051/parasite/2022050

