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Associations between toxoplasmosis and rheumatoid arthritis among patients in Zakho City, Kurdistan Region/Iraq

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Sarwin Sultan Muhamad Mizuri [1*] Chira Abdulla Ramadhan [1] Wijdan Mohammed Salih Mero [1]

Abstract

Background and objective: Toxoplasmosis is a zoonotic disease that can cause a wide spectrum of clinical diseases. This study aimed to investigate the association between toxoplasmosis and rheumatoid arthritis among patients, besides, to confirm the role of *Toxoplasma gondii* in the enhancement and modulation of autoimmune diseases such as rheumatoid arthritis.

Methods: A cross-sectional study was implemented from August 2021 to February 2022, in which blood samples were taken conveniently from a total of 180 patients from both genders and different ages (15-60 years). These patients attended Zakho General Hospital and some private clinics suffering from symptoms related to rheumatoid arthritis. All samples were tested for *Toxoplasma gondii* immunoglobulins G and M (IgG, IgM) antibodies, C- reactive proteins (CRP), rheumatoid factor (RF), and erythrocyte sedimentation rate (ESR).

Results: The total seropositivity of anti-*Toxoplasma gondii* IgG and IgM Abs using ELISA were 13.9% and 3.9%, respectively. Furthermore, the highest rate (16.4%) of IgGAbs was recorded among the male group, while, the highest rate (4.8%) of IgMAbs, was recorded among the female group. Furthermore, the highest rate of IgG, and IgM Abs were recorded among the age group 46-60 in both genders (20%, 5.7% respectively). In addition, the statistical analysis demonstrated the presence of significant relationship between IgG Abs with CRP and RF test results (P = 0.027, 0.039 respectively). Whereas, concerning ESR only seropositive IgM Abs results were statistically significant (P = 0.049).

Conclusion: Toxoplasmosis may influence the immune system and act as a contributing factor in the exacerbation of autoimmune illnesses, including rheumatoid arthritis. Subsequent investigations will be essential to delineate the risks of T. gondii within the human population.

Keywords: Toxoplasmosis; Rheumatoid Arthritis; CRP; RF; ESR.

Introduction

Most mammals' including human serves as intermediate hosts for *T. gondii* whereas, members of the family Felidae including the domestic cats serves as final hosts. The occurrence of toxoplasmosis is influenced by numerous factors such as gender, age, cultural levels, hygienic measurements, residency, eating habits and contacts with cats.[1] It is supposed that *T. gondii* has a major role to generate and stimulate the autoimmune disorders among heritably susceptible persons through epitope dispersion, molecular mimicry, or bystander activation. Moreover, there is a correlation between toxoplasmosis and autoimmune infections like rheumatoid arthritis, since it's considered as a risk factor for such diseases, which were also revealed in numerous investigations.[2-5] Alternatively, the common autoimmune disorders including rheumatoid arthritis, which cause inflammation of joints and is responsible of several medical condition

[1] Department of Biology, Faculty of Science, Zakho University, Duhok, Iraq.
Correspondence: sarwin.muhammad@uoz.edu.krd

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that affects about 0.5-1% of peoples. The infection grants with systematic effects, formation of autoantibodies (rheumatoid factor), and swollen joints.[6] Theoretically, arthritis is named a degenerative joint disease. Arthritis consists of two major kinds (non-inflammatory and inflammatory) arthritis. Gout disorders and RA are examples for inflammatory, whereas, osteoarthritis is an example for the non-inflammatory. Differentiation among them can be influenced by location, time, and joint pain, in addition to the occurrence of enlargement and exterior symbols of joint, for instance rash.[7]

As, tachyzoites are extremely spreadable, if the immune system is weak, they can't be controlled and can turn into generalize toxoplasmosis that is fatal.[8] Due to the infection specific anti-*Toxoplasma* antibodies were created in a consequence of a strong combination between humoral response and Th1 cytokine profile. The basic incident in the initiation of immunity against *T. gondii* is the prompt action of the type one inflammatory cytokine reaction. For this reason, that polymorphonuclear leukocytes rapidly responds to the infection by departing the peripheral blood and aggregating in the infection site.[9]

Regarding to parasite-host association, the parasite may seek to encourage strong defensive immunity of the host; in order to deprive from such a reaction, the parasite speedily overwhelms the host and kill it. By this mechanism, the chances of parasite transmission to other hosts are minimized.[10,11] Furthermore, Arthritis can be diagnosed in the Laboratory by using different laboratory assessments such as: antibody test of anti-DNA-systemic lupus erythematosus (SLE), rheumatoid factor, antinuclear factor (ANA), C. reactive protein (CRP) determination, and (anti-CCP) anticyclic citrullinated peptide. Besides, anti-cardiolipin (ACL), antiphospholipid (APL) adding to the calculations the level of calcium, uric acid, and inorganic phosphate in the serum.[12] The role of *T. gondii* in the improvement of arthritis sustained through numerous studies. The association between arthritis and toxoplasmosis was noticeable from a study performed among Chinese inhabitants (820 individuals participated, including control and patients) by using ELISA technique. Also, the large quantity of IgG of *Toxoplasma* antibodies was established plentiful among patient's distresses with arthritis (18.8%) while among healthy persons only 12% was recognized. Patients suffered with arthritis and rheumatoid arthritis displays a great seroprevalence rate with anti-*Toxoplasma* antibodies than osteoarthritis and gouty arthritis.[13]

Alternative study performed among rheumatoid arthritis patients in Egypt, they revealed the positive relationship of seropositivity of *T. gondii* not only on the presence of the disease, moreover, increased medical indications, comprising of swollen joints, higher number of tender, prolong disease duration, and extended morning difficulty.[14] *Toxoplasma gondii* have the ability to invade cervical lymph nodes, causing lymphadenopathy several times correlate stiffness of cervical, or migration of parasite through lymphatic system and blood stream may cause serious health problems of fetus and neonates by attacking another organs for instance eye orbit, muscles and brain which commonly causes miscarriage, hydrocephaly or microcephaly, encephalitis, abnormal sight plus myositis correspondingly.[15] Currently, few studies were achieved in this concern in Iraq specifically in Zakho City. Therefore, the current study was implemented to find out the possible correlation between toxoplasmosis and rheumatoid arthritis in addition to its role in immune response modulation among patients who attended Zakho general Hospital and some private clinics in Zakho City/ Kurdistan Region, Iraq.

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Methods

Study design: Across sectional study was performed from August 2021 to February 2022. A total of 180 patients, comprising both genders and ranging in age from 15 to 60 years, were conveniently selected for the study, all of them were afflicted with rheumatoid arthritis. The enrolled patients visited Zakho General Hospital and many private clinics in Zakho City, just a few kilometers from the Iraqi–Turkish border.

Sample Collections and Laboratory Examinations

Five ml of venous blood was drained from each contributor after taking a verbal consent and an approval from General directorate of Health in Zakho City. Each blood sample was divided into two parts, one was placed in a fully labeled tube with patient's full information containing anticoagulant for ESR test, which represent the prevalence frequency of inflammation due to toxoplasmosis and rheumatoid arthritis using the Westergren's tube method (China) and the obtained data were compared with relevant control values. While the second portion of the blood was placed in another labeled tube comprising clot activator used for serum separation after that centrifuged at 4000 rpm for 5 minutes and used for serological tests. Each serum sample was kept in 2-ml Eppendorf tube; all sample tubes were stored in deep freezer at −20 °C in the laboratory for further processing.

The samples were tested to detect *T.gondii* antibodies (IgG, IgM) using ELISA kits (Bioactive diagnostica/Germany), C-reactive proteins (CRP) and Rheumatoid Factor (RF) were determined using titerkits from MISPA-i_3/ AGAPPI DIAGNOSTIC (Switzerland GmbH) both tests display positive results in response to inflammation, all procedures were performed according to the instructions provided with each test kit.

Statistical Analysis

For data analysis software package of social science SPSS program (version 26) was used. Frequency and frequency percentage tables implemented for describing the data. Besides, Chi-squared test was used for detecting the association between *Toxoplasma* seropositivity and other variables. The P-value equal to or less than 0.05, was deemed statistically significant.

Ethics consideration

Verbal consent was obtained from enrolled patients, and the study received approval from the ethical committee of the University of Zakho and Zakho General Hospital, Zakho, Kurdistan Region, Iraq (No.614/5, in 11/8/2021).

Results

Demographic Characteristics

The total number of participants was 180 and the total seropositive rate of anti-*Toxoplasma* IgG and IgM Abs using ELISA were 13.9%, 3.9%, respectively, as shown in Table 1.

Table 1 Total distribution of anti-*Toxoplasma gondii* antibodies using ELISA

Anti-*Toxoplasma* antibodies		No.	%
IgG	Positive	25	13.9
	Negative	155	86.1
IgM	Positive	7	3.9
	Negative	173	96.1
Total		180	100.0

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The majority (69.4%) of them being females (125/180). Moreover, the maximum rate for ELISA IgG Abs was documented among males as compared to females, while, the maximum rate of IgM Abs was recorded among females. Statistically the differences between the rates of both types of antibodies among both genders was non-significant (*P* >0.05) as indicated in Table 2.

As regarding to age, the age group 46-60 showed the highest rates of both antibodies among both females and males (Table 3).

The correlation of C-reactive protein with anti-*Toxoplasma* IgG and IgM Abs, indicated the presence of positive correlation between both parameters, since the maximum seropositivity rate of ELISA IgG, IgM Abs (22%, 5.1%), respectively were verified among patients with positive C-reactive protein, with statistically significant (*P* <0.05) difference for IgG Abs only as shown in Table 4.

Table 2 Distribution of toxoplasmosis in relation with gender

Gender	Total No.	IgG Positive		*P*-value*	IgM Positive		*P*-value*
		No.	%		No.	%	
Males	55 (30.6%)	9	16.4	0.524	1	1.8	0.677
Females	125 (69.4%)	16	12.8		6	4.8	
Total	180	25	13.9		7	3.9	

*Chi-Squared Test

Table 3 Distribution of toxoplasmosis regarding to Age group

Age in years	Total No.	IgG Positive		*P*-value*	IgM Positive		*P*-value*
		No.	%		No.	%	
15 – 30	75	10	13.3	0.480	4	5.3	0.410
31 – 45	70	8	11.4		1	1.4	
46 – 60	35	7	20.0		2	5.7	
Total	180	25	13.9		7	3.9	

*Chi-Squared Test

Table 4 Correlation between *Toxoplasma gondii* distribution and C- reactive protein (CRP)

C-reactive Protein	Total No.	IgG Positive		*P*-value*	IgM Positive		*P*-value*
		No.	%		No.	%	
Positive	59	13	22.0	0.027	3	5.1	0.685
Negative	121	12	9.9		4	3.3	
Total	180	25	13.9		7	3.9	

*Chi-Squared Test

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Similarly, regarding to the correlation between RF patients, and toxoplasmosis, also the maximum rates of anti-*Toxoplasma* IgG and IgM Abs were observed among those patients with positive RF, statistically there was significant (P <0.05) relation for IgG Abs only (Table 5).

Correspondingly, patients with high levels of ESR, showed the highest rates of Anti-*Toxoplasma* IgG and IgM Abs (16.1%, 8.1%), respectively. Statistical analysis indicated the presence of significant difference (P <0.05) in the rate of IgM Abs among both groups (Table 6).

Discussion

In Zakho City considerable numbers of patients were referred to the outpatient clinics and some of them visited private clinics complaining from joint pains and other arthritis symptoms. These observations encouraged us to conduct this study in order to find if there is any significant correlation between toxoplasmosis and these symptoms. Since, some studies linked between severe clinical signs for instance morning stiffness, higher tender and enlarged joints represented among seropositive toxoplasmosis and rheumatoid arthritis patients than toxoplasma seronegative patients.[2] This parasitic infection leads to immunomodulatory variable influences. Among humans, toxoplasmosis leads to proportioned polyarthritis of the joints of fingers, hands, knees and wrists.[16]

Among both genderin this study, the seropositivity rate of ELISA IgG Abs was much higher than the seropositive of IgMAbs (13.9%, 3.9%) respectively. While, in Kirkuk city, Salman and Mohammed examined 99 serum samples and recorded a seropositivity rate of 54.09%, comprising 47.54% for IgM and 6.55 %for IgG Abs.[17] Likewise, a higher prevalence was reported in other studies, which were 11.58%, 0.63%in Zakho City and 36.7%, 1.17%in Kirkuk City for ELISA IgG and IgM, Abs respectively.[1,18] In the present

Table 5 Correlation between *Toxoplasma gondii* distribution and Rheumatoid factors (RF)

RF	Total No.	IgG Positive		*P*-value*	IgM Positive		*P*-value*
		No.	%		No.	%	
Positive	15	5	33.3	0.039	1	6.7	0.462
Negative	165	20	12.1		6	3.6	
Total	180	25	13.9		7	3.9	

*Chi-Squared Test

Table 6 Correlation between *Toxoplasma gondii* distribution and erythrocyte-sedimentation rate (ESR)

ESR	Total No.	IgG Positive		*P*-value*	IgM Positive		*P*-value*
		No.	%		No.	%	
High	62	10	16.1	0.529	5	8.1	0.049
Normal	118	15	12.7		2	1.7	
Total	180	25	13.9		7	3.9	

*Chi-Squared Test

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concerning to IgG Abs (16.4%), conversely, the maximum rate of IgM Abs (4.8%) were seen among females. This outcome contradicts with the study achieved in Kirkuk city which documented maximum rate of IgG and IgMAbs using ELISA among females which were 48.53%, 6.62%, respectively.[17] The variation in these rates may be due to sample sources. Regarding to the age, the maximum rate was recorded among the age group 46-60 years which was 20.0%, and 5.7% for IgG and IgMAbs, respectively. This result contradicts with the previous study performed in Zakho city that revealed the highest rate of anti-*Toxoplasma* Abs among the age group 33-38 years which were 20.43%, 1.07% for IgG and IgMAbs, respectively.[1] This dissimilarity may be attributed to the type of samples, because in the present study both genders were enrolled, while the previous study dealt with females only. Correspondingly, rheumatoid arthritis can be detected by a potential marker, the C-reactive protein (CRP), it is a sensitive test, as its level in the blood is elevated during rheumatoid arthritis.[19]

Furthermore, the highest rate of anti-*Toxoplasma gondii* IgG and IgMAbs (22.0%, 5.1%) were recorded among patient who displayed positive for C-reactive protein as well, with detection of statistically significant difference between seropositive and seronegative CRP patient correlated to ELISA IgG test. This result is in line with a study achieved in Egypt in which high rate of CRP also showed high seropositivity rate for ELISA IgG Abs (99.9%) and they attributed it toparasitic infections that elicited immune system modulation.[14] In addition, the maximum seropositive rate of IgG and IgM Abs were recorded among patients who were positive of RF test (33.3%, 6.7%) respectively. Also, significant differences were seen among ELISA IgGAbs and RF outcomes. This consequence agree with the study implemented in Thi-Qar Province/Iraq, by Al-Oqaily and Al-Ubaidi, who stated that all patients with toxoplasmosis displayed positive result for RF test.[20] Besides, rheumatoid arthritis patients usually have anti-cyclic citrullinated peptide and circulating auto-antibodies characterized by rheumatic factor and these auto Abs are significantly associated with *T. gondii* mainly when they are of high quantities.[21] The consequences were suggested in Table 6 which showed high rate (16.1% and 8.1%) of *Toxoplasma* IgG and IgM Abs in individual with high rate of ESR. As regards to both genders ESR rates were above than normal limits, with a significant difference (P <0.05) among ELISA IgM Abs and ESR results. This outcome agrees with the study performed in Egypt that revealed the maximum seropositivity of anti-*Toxoplasma* Abs among patients with high ESR level as compared to those with low levels of ESR.[14] Similarly, our finding agrees with the study achieved in Kirkuk city they recorded high ESR levels among seropositive cases.[17] These achieved outcomes based on several medical and experimental studies that reported that infection with microbes has the ability to encourage and/or amplify the signs of arthritis.[18]In addition, the ESR value has significant relationships with gender and age, since its level is increasing with age and the gender, besides females are more susceptible thanmales.[22]

Conclusion

We can conclude from this study that, there is a link between toxoplasmosis and other human diseases due to the nature of the parasite which is mainly controlled by host immune status. Moreover, primary or secondary infection with *T. gondii* parasite possibly will motivate immune response and modify the immune response to prompt autoimmune disorders. This study outlined the significant influence of *T. gondii* on different autoimmune tests among rheumatoid arthritis patients such as CRP, RF and ESR and their correlation with gender and age. Therefore, to get a good insight to this situation, more detailed investigations are crucial to identify the

relationship between toxoplasmosis and other human diseases which can be valuable for improving the treatment and controlling the disease.

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Competing interests

The authors declare that they have no competing interests.

References

1. Sultan S, Mero WMS. Seroprevalence of anti-*Toxoplasma gondii* antibodies among women of childbearing age in Zakho City, Kurdistan Region/ Iraq. Zanco J Pure Appl Sci 2020; 32(3):75–84. http://dx.doi.org/10.21271/zipas.
2. El-Sayed NM, Kishik, SM, Fawzy RM. The current status of *Toxoplasma gondii* infection among Egyptian rheumatoid arthritis patients. Asian Pac J Trop Dis 2016; 6(10):797–801. https://doi.org/10.1016/S2222-1808 (16)61133-7.
3. Mirsaeedi H, Fereidunian A, Mohammadi-Hosseininejad SM, Dehghanian P, Lesani H. Long-term maintenance scheduling and budgeting in electricity distribution systems equipped with automatic switches. IEEE Trans Industry Inform IEEE T IND INFORM 2017; 14(5):1909–19. https://doi.org/10.1109/TII.2017.2772090.
4. Shakir OY, ALQadhi BN. The impact of *Toxoplasma gondii* infection on the level of shla-G and its receptor in Iraqi patients infected with rheumatoid arthritis. Ann Romanian Soc Cell Biol 2021; 25(6):6994–7001. https://annalsofrscb.ro/index.php/journal/article/view/6815.
5. Dreaj HA, Hathal HD, Hassan Abbas AA. Seropositivity of *Toxoplasma gondii* antibodies in rheumatoid arthritis patients under treatment with TNF-? IABMS 2022; 42(2):314–7. https://biomedicineonline.org/index.php/home/article/view/934.
6. Hosseininejad Z, Sharif M, Sarvi S, Amouei A, Hosseini SA, Chegeni TN, et al. Toxoplasmosis seroprevalence in rheumatoid arthritis patients: a systematic review and meta-analysis. PLoS Negl Trop Dis 2018; 12(6). https://doi.org/10.1371/journal.pntd.0006545.
7. Zhu Y, Pandya BJ, Choi HK. Prevalence of gout and hyperuricemia in the US general population: the National Health and Nutrition Examination Survey 2007–2008. Arthritis Rheumatol 2011; 63 (10):3136–41. https://doi.org/10.1002/art.30520.
8. Al-Malki SE. Toxoplasmosis: stages of the protozoan life cycle and risk assessment in humans and animals for an enhanced awareness and an improved socio-economic status. Saudi J Biol Sci 2021; 28(1):962–9. https://doi:10.1016/j.sjbs.2020.11.007
9. Petríková J, Jarčuška P, Pella D. Chapter 37 - Toxoplasma and autoimmunity. Edited by: Yehuda Shoenfeld, Nancy Agmon-Levin and Noel R. Rose. Infection and Autoimmunity (Second Edition). Academic Press 2015; 643–56. https://doi.org/10.1016/B978-0-444-63269-2.00044-1
10. Sasai M, Yamamot. M, Innate, adaptive, and cell -autonomous immunity against *Toxoplasma gondii* infection. Exp Mol Med 2019; 51:1–10. https://doi.org/10.1038/s12276-019-0353-9
11. Abi Abdallah DS, Lin C, Ball CJ, King MR, Duhamel GE, Denkers EY. *Toxoplasma gondii* triggers release of human and mouse neutrophil extracellular traps. Infect Immun 2012; 80(2):768–77. https://doi.org/10.1038/s12276-019-0353-9
12. Gaddy JR, Vista ES, Robertson JM, Dedeke AB, Roberts VC, Klein WS, et al. Rheumatic disease among Oklahoma tribal populations: a cross-sectional study. J Rheumatol 2012; 39(10):1934–41. https://doi.org/10.3899/jrheum.110984
13. 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. Infect Dis Poverty 2017; 6(1):1–7. https://doi.org/10.1186/s40249-017-0367-2
14. 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. https://doi.org/10.1007/s00296-017-3703-8
15. Fallahi SH, Abdolmohamad R, Nourollahpour SH, Hamed B, Paktinat S. "An updated literature review on maternal-fetal and reproductive disorders of *Toxoplasma gondii* infection. J Gynecol Obstet Hum 2018; 47(3):133–40. https://doi.org/10.1016/j.jogoh.2017.12.003
16. Sultan S, Mero WM. Serodiagnosis of toxoplasmosis by using ELISA and IgG avidity test in relation to some risk factors among women at childbearing age in Zakho City, Duhok Province/Iraq. QJPS 2021; 26(4):1–8. https://doi.org/10.29350/jops. 2021.26. 4.1331
17. Salman YJ, Mohammed KA. Relationship between *Toxoplasma gondii* and arthritis among patients in Kirkuk city. Int J Curr Res Aca Rev 2015; 3(8):175–87.
18. Al-Aubaidi IK, Al- Oqaily MA, Hamad SS. Role of interleukin 33 during infection with toxoplasmosis in rheumatoid arthritis patients. Indian J Forensic Med Toxicol 2020; 14(1):526–31. https://doi.org/10.37506/ijfmt.v14i1.101
19. Pope JE, Choy EH. C-reactive protein and implications in rheumatoid arthritis and associated comorbidities. Seminars in Arthritis and Rheumatism 2021; 51(1):219–29. https://doi.org/10.1016/j.semarthrit.2020.11.005
20. 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. IJSN 2017; 8(4):824–9.
21. Wang L, Ding Y, Guo X, Zhao Q. Role and mechanism of vascular cell adhesion molecule-1 in the development of rheumatoid arthritis. Exp Ther Med 2015; 10(3):1229–33. https://doi.org/10.3892/etm.2015.2635
22. Singh G.C-reactive protein and erythrocyte sedimentation rate: Continuing role for erythrocyte sedimentation rate. ABC 2014; 4 (1):42706–5. http://dx.doi.org/10.4236/abc.2014.41002