INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

PREVALENCE OF TOXOPLASMOSIS IN IRAQI RHUMATOID ARTHRITIS PATIENTS AND DETECTION LEVELS OF MCP-1 AND TGF-β CHEMOKINES DURING INFECTION

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ABSTRACT

Toxoplasma gondii is an intracellular protozoan parasite that has recently a relationship with autoimmunity and induces a variable immunomodulatory effects. The present study was included 258 patients with rheumatoid arthritis under treatment with methotrexate underwent to Al-Kindi and Baghdad Teaching Hospitals in Baghdad province, Iraq and 50 healthy individuals considered as control group. The samples were collected during the period from September 2016 to the end of February 2017 .In order to diagnose RA, agglutination rheumatoid factor test (RF), C- reactive protein test (CRP) and ESR test were used. ELISA technique was applied for diagnosis toxoplasmosis. The percentage of *T. gondii* positivity IgG in rheumatoid arthritis (RA) patients was (36.82%) ,While percentage of *T. gondii* IgM was (1.55%) . High levels of MCP-1 and TGF- 1 were recorded in infected groups RA with toxoplasmosis and RA only 298.018±82.94pg/ml and 3.29± 1.07 pg/ml respectively with a significant differences when compared to control group (P<0.001).

KEYWORDS*: Toxoplasmosis,* Rheumatoid Arthritis (RA), MCP-1, TGF- 1.

INTRODUCTION

T. gondii is an intracellular protozoan parasite that infects approximately all warm-blooded animals, including humans, and is considered one of the most effective eukaryotic pathogens[1]. Around 30 % of human population globally have a chronic infection infected with *T. gondii*[2]. It has a complex life cycle with asexual reproduction taking place in different tissues of mammals such as (human) and birds that is consider intermediate hosts and sexual reproduction that occur in digestive epithelium of cats and ultimate the final host [3]. Humans become infected in one of three ways: via ingesting *T. gondii* tissue cysts (comprising bradyzoites) that founded in the undercooked meat (particularly beef and pork) of infected food animals; *via* ingesting highly infectious oocysts (comprising sporozoites) that found in water, garden soil, children's sand boxes, *etc*…. additionally polluted via infected cat feces; or during congenital transplacental transmission of quickly replicating tachyzoites from mothers who become infected during pregnancy and pass the infection to the fetus. After proliferation of tachyzoites in different organs through the acute phase, the parasite forms cysts preferentially in the brain and creates a chronic infection [4]. It is one of the main opportunistic infection agent in immune compromised cases for example haemodialytic, leukemic, Hodgkin's and non-Hodgkin's disease and diabetic patients, also its association with RA [5-7]. Rheumatoid arthriti is a chronic, immunological disease characterized via symmetrical inflammation in the joints leading to pain, swelling and stiffness of the joints, eventually resulting in joint destruction, incapacity and weak quality of life [8]. It

is hypothesised that genetic and environmental factors play a role in the manifestation of RA. The major genetic danger factor is thought to be the human leukocyte antigen (HLA DRB1) alleles, while infection with viruses, hormones or bacteria are some of the identified environmental activates, This hypothesis, however, is still inconclusive [9]. The aim of this study was to evaluate serum levels of chemokines (MCP-1and TGF-) in Rheumatoid arthritis patients infected with toxoplasmosis, and to assess the correlation of these chemokines with clinical and laboratory parameters like Rheumatoid Factor (RF), C-Reactive Protein (CRP), Erythrocytes sedimentation Rate (ESR).

MATERIALS & METHODS

This study was included 258 samples of patients with rheumatoid arthritis treated with methotrexate attending to Department of Rheumatology in Al-Kindi and Baghdad Teaching Hospitals in Baghdad province. During the period from September 2016 to the end of February 2017. Their age ranged from 13-68 years, in addition a group of 50 healthy persons after presenting them to a specialist in joint diseases were enrolled in this study as a control group. Five ml of venous blood were collected from each subjects (patients and control) groups and placed in gel tube, the serum was separated and divided in eppendorff tubes then stored at -20C° until it is used.

Serological tests

1- Agglutination Rheumatoid Factor Test (RF):

Detection of antibody-antigen interactions directly in human serum, the kit is supplied by (Biosysteme, Spanish).

2- C- Reactive Protein test (CRP)
This test is based on the principle of Agglutination of quantitative and semi-quantitative detection of CRP in human serum; the kit is supplied by (Spinract, Spanish).

3- ELISA *T. gondii* –IgG/ IgM:
The ELISA Toxo-IgG / IgM (From Bioactiva kit, Company, Spain) is an IgG/IgM- capture immune enzimatic assay for the determination of IgG/IgM antibodies against *T. gondii* in the patient's serum or plasma.

Calculation of results
The qualitative result was calculated by dividing the absorbance value of each sample by the value of the cut – off point:
Positive sample: If the ratio ≥ 1.1.
Negative sample: If the Ratio < 0.9.
According to the ELISA result the studied groups were divided to (99) Rheumatoid arthritis patients infected with toxoplasmosis and (159) Rheumatoid arthritis patients not infected with toxoplasmosis.

4- Serum Level of Cytokines:
Serum levels of MCP-1 and TGF- were measured by using specific enzyme-linked immunosorbent assay (ELISA) kit ((Peprotech, USA), according to the manufactures protocol.

Statistical Analysis
Statistical Analysis System- SAS (2012) program was used for data analysis. The data are expressed as percentage (%) and mean ± standard deviation (SD) and standard error (S.E.). Differences between means were assessed by least significant difference –LSD test. At the probability level of 0.05 and 0.01 to assess the differences between the study groups.

RESULTS
Serum samples were analyzed using ELISA to detect IgG and IgM antibodies of *Toxoplasma gondii* parasite, table (1) shows number of positive cases oF IgG in 95 patients with RA in 36.82% compared to 163 negative cases at 63.18%, while the control group was 100% negative. High signefecant differences were recorded between studied groups (p ≤ 0.001).

TABLE 1: Distribution of the studied groups according to the presence of IgG. with its significance comparisons

Parameter	Response	Rheumatoid Arthritis		Control		P-value
		No.	%	No.	%	%
IgG Antibody	Positive	95	36.82	0	0.00	0.0001 **
	Negative	163	63.18	50	100	
Total		258		50		
P-value		0.0001 **				

Table (2) shows the comparisons of IgG levels in all studied groups estimated by IU/Ml, the level of IgG is high in the group of rheumatoid arthritis patients with toxoplasmosis (0.395 ±0.18) compared to rheumatoid arthritis patients without toxoplasmosis (0.079 ±0.05) and control group (0.0674 ±0.04), these results indicate highest and lowest response.

TABLE 2: Descriptive statistics of IgG levels estimated by IU/ml for studied groups

Groups	No.	Mean	Std. Dev.	Std. Error	Lower Value	Upper Value
RA with toxoplasmosis patients	95	0.395	0.18	0.03	0.184	0.9
R.A patients	163	0.079	0.05	0.003	0.007	0.176
Control	50	0.0674	0.04	0.003	0.002	0.156
LSD Value	0.1087 **					
P-value	0.0026					

Table (3) shows IgM levels in studied samples, with only four positive cases of 258 patients in 1.55% and 254 negative cases at 98.44%, while the control group was completely negative. The statistical analysis did not record any significant differences between all the studied groups.

TABLE 3: Distribution of the studied groups according to the presence of IgM. with its significance comparisons

Parameter	Response	Rheumatoid Arthritis		Control		P-value
		No.	%	No.	%	%
IgM Antibody	Positive	4	1.55	0	0.00	1.00 NS
	Negative	254	98.44	50	100	
Total		258		50		0.0001 **
P-value		0.0001 **				

Table (4) showed the mean values of IgG in all the groups ,rheumatoid arthritis patients with toxoplasmosis has registered the highest value 0.540 ± 0.062 IU/ml then rheumatoid arthritis patients 0.0810 ± 0.022 IU/ml finally control group has 0.063 ± 0.018 IU/ml, also the table was referred to the highest and lowest response of IgM levels. Statistical analysis showed high significant differences between the groups.

TABLE 4: Descriptive statistics of IgM levels estimated by IU/ml for studied groups

Groups	No.	Mean	Std. Dev.	Std. Error	Lower Value	Upper Value
RA with toxoplasmosis	4	0.540	0.062	0.029	0.241	0.712
R.A patients	254	0.0810	0.022	0.003	0.002	0.178
Control	50	0.063	0.018	0.006	0.001	0.152
LSD	0.194 **					
P-value	0.00061					

Table (5) showed the   distribution of studied groups according to the results of RF, CRP tests for diagnosis of rheumatoid arthritis, patients of RA were completely have positive responses, while control group was completely negative, complete passivity with highly significant differences p 0.0001.

TABLE 5: Values of rheumatic factor and C- Reactive protein tests of the studied groups

Tests	Response	RA with toxoplasmosis		Rheumatoid Arthritis		Control		p-value
		No.	%	No.	%	No.	%	
RF	Ve+	99	100	159	100	0	0.00	0.0001 **
	Ve-	0	0.00	0	0.00	50	100	
CRP	Ve+	99	100	159	100	0	0.00	0.0001 **
	Ve-	0	0.00	0	0.00	50	100	
Total		99		159		50		

Table (6) referred to the comparison of the results of ESR test for all groups that registered, high significant level in sera of RA patients with toxoplasmosis 53.82 ± 0.67 compared to rheumatoid arthritis only 40.87 ± 1.29 and control group 9.42 ± 0.34.

TABLE 6: Results of ESR test in studied groups

Groups	NO.	Mean± Std. Error ESR
RA with toxoplasmosis	99	53.82±0.67 a
RA patients	159	40.87± 1.29 b
Control	50	9.42 ± 0.34
LSD value	---	5.997**
P-value	---	0.0001
**P<0.01.		

Table (7) showed the mean values of the levels of the chemo attractant MCP-1 and the group of rheumatoid arthritis patients with toxoplasmosis registered a higher rate of disease (298.018±82.94) compared with a group of rheumatoid arthritis patients only (222.839 ± 86.85) and control group (66.973 ± 21.51), the table also indicates the highest and the lowest value for the MCP-1 and for all studied groups.

TABLE 7: Levels of MCP-1 chemoattractant estimated in pg/ml for studied groups

Groups	No.	Mean	Std. Dev.	Std. Error	Lower Value	Upper Value
RA with toxoplasmosis	50	298.018	82.94	12.50	170.156	560.531
RA patients	50	222.839	86.85	19.42	92.341	356.113
Control	50	66.973	21.51	4.81	32.819	106.344
P-value	0.0001					
LSD	55.975 **					

TABLE 8: Multiple comparisons of the MCP-1 concentrations (pg/ml) according to LSD test for potential couples between studied groups

Parameter	Group(1)	Group(j)	Mean Diff.	P-value	Sig.
MCP-1 Concentration (Pg/ml)	RA with toxoplasmosis	RA patients	75.179	0.0712	NS
		Control	231.045	0.0001	HS
	RA patients	Control	155.866	0.0002	HS

Table (8) referred   to the differences of the means for MCP-1 among all studied groups, the results didn't record significant difference when comparing the level of MCP-1 in rheumatoid arthritis patients with toxoplasmosis and the group of rheumatoid arthritis only, while high significant

differences at probability of P<0.01 were recorded when comparing the previous groups with the control group.
Table (9) showed high level of TGF- 1 in the group of rheumatoid arthritis patients with toxoplasmosis 3.29 ± 1.07 compared to rheumatoid arthritis patients 1.36 ± 0.29 and control group 0.182 ± 0.05.

TABLE 9: Levels of TGF- 1 estimated in pg/ml for studied groups

Groups	No.	Mean	Std. Dev.	Std. Error	Lower Value	Upper Value
RA with toxoplasmosis	50	3.29	1.07	0.16	1.11	4.88
RA patients	50	1.36	0.29	0.06	0.85	1.98
Control	50	0.182	0.05	0.01	0.092	0.279
P-value	0.0001					
LSD	1.052 **					

Table (10) shows the comparisons in the means of the cytokine TGF- 1 among all studied groups, significant differences (P < 0.05) were registered when comparing the values of TGF- 1 for the patient's in rheumatoid arthritis with toxoplasmosis and rheumatoid arthritis only, while highly significant differences at probability of P < 0.01 was recorded when compared to the control group. TGF -1 levels of rheumatoid arthritis patients and control group showed significant difference of P < 0.05.

TABLE 10: Multiple comparisons of TGF- 1 levels according to the LSD test for potential couples between studied groups

Parameter	Group(1)	Group (j)	Mean Diff.	P-value	Sig.
TGF- Concentration (pg/ml)	RA with toxoplasmosis	RA patients	1.93	0.0277	S
		Control	3.108	0.0052	HS
	RA patients	Control	1.178	0.0394	S

DISCUSSION

Numerous reports investigated the relationship between autoimmune diseases as RA and *T. gondii* in order to evaluate the role of this parasitic infections in triggering the immnopathological reactions [6,7,10,11]. In the present study, the association between rheumatoid arthritis and toxoplasmosis in Iraqi patients has been identified through the detection of antibodies IgG and IgM in sera of the studied groups the ELISA test, it is one of the beast sensitive tests for the detection of the toxoplasmosis infection (12). The current study is agree with Kuba *et al*. [5] which found that the percentage of the IgG and IgM were 33.33% and 20.40% respectively in RA patients treating with methotrexate, also it is agreement with Salman and Mohammed[7] which observed that the percentage of IgG was (47.54%) and IgM was (6.55%) in RA patients. Tian *et al*.[13] who revealed that the prevalence of anti-*T. gondii* IgG in arthritis patients and control were (18.8%) and (12%) respectively whereas the prevalence of IgM Ab in arthritis patients and control were (1.5%) and (1.2%) respectively these results were similar to the present results .The reason for this rise of the incidence of infection is assumed to be the outcome of different culinary practices and ability of oocysts to survive in different climates as well as having a close relationship with cats [14]. An increased danger of *T. gondii* infection in RA patients perhaps anticipated due to the disease linked immunological variations that compromise adaptive cellular immunity - crucial for the control of an intracellular parasite *T. gondii*. RA is related with alterations in the T cell repertoire [15]. The rheumatic factor was used as a marker for rheumatoid arthritis and it is included in the criteria for classification of the disease for more than half a century [16]. The results of the test rheumatoid factor were compatible with El-Sayed *et al*. [10]

who found the proportion of patients of 54 rheumatoid arthritis with toxoplasmosis was 83%, while the percentage of patients without toxoplasmosis was 78% represented 46 patients. There is another potential marker for increasing the danger of rheumatoid arthritis, which is a C-reactive protein (CRP), it is a sensitive test and their level was increase in rheumatoid arthritis patients, and it is also associated with the response to treatment that may reduce or remain normal in rheumatoid arthritis patients after effective treatment [17]. The results of the present study were agree with Salih[18] which conducted a study for women in the Kurdistan region suffering from rheumatoid arthritis and all samples were positive for the RF and CRP tests. Patients with rheumatoid arthritis typically have circulating auto-antibodies represented by rheumatic factor and anti-cyclic citrullinated peptide. These auto antibodies are associated with a significance close relationship with *T. gondii* especially when they are of high rates [19,20]. The ESR test is one of the beast sensitive tests for many inflammations types as the rheumatoid arthritis and systemic lupus erythematous and other diseases [21]. The results of the current study recorded that agreement with El-Sayed *et al*. [10] who showed the ESR test rate was high RA patients with toxoplasmosis of 54 patients, while Fischer *et al.* [6] register the mean of ESR test in rheumatoid arthritis patients with seropositive toxoplasmosis is 43.18 mm/h but the patients who showed seronegative toxoplasmosis, is 36.08mm/h, and this is agree with the current study. As well as, the ESR test is linked to age and sex factors, as its value increases with age and has the highest value for women over men [22]. Monocyte chemoatrractant protein (MCP-1) it is a class of cytokines (chemokine mediate) with functions that include attracting white blood cells (Leukocytes) to sites of infection, as it is regulates the migration of phagocytes,

natural killer cells and T-cell that induce of various diseases including multiple sclerosis, rheumatoid arthritis and atherosclerosis [23,24]. The results of the current study showed that the highest level of this chemoattractant was in the group of patients with rheumatoid arthritis infected in toxoplasmosis this may be due to that MCP-1 participate in recruiting cells and activation of many leukocytes such as monocytes, phagocytes and multinucleated cells as well as infiltration of lymphocytes into a site inflammation to eliminate parasite [25]. The current study disagree with Ali [26] which found the low levels of MCP-1 were observed in pregnant women with acute toxoplasmosis because the tachyzoites induce the immune response to infection will stimulate the phagocytes and lymphocytes that act the production of MCP-1[27]. The present study is agree with Slaovi *et al*. [28] that recorded high-level MCP-1 in serum and synovial fluid with high significant differences ($P < 0.01$) in a group of patients of strong active rheumatoid arthritis compared with the mild active. TGF- 1 is a pleiotropic chemoattractant that organizes many biological functions starting from growth and urging cells on reproduction and differentiation, also have the ability to induce apoptosis [29]. The current study showed a rise in the concentration of TGF- 1 in patients of RA with toxoplasmosis compared with other groups. This chemoattractant plays a prime role in organizing functions of immune cells also inhibited T and B lymphocytes and induce balance[30]. This chemoattractant also contributes to the reorganization of the tissue response after the infection occurrence in addition to participate in the development of Th17 cells and lymphocytes that plays a major role in stimulating and inhibiting immune response against parasite infection (31). The current study has been agree with Ali [26] that has shown a significant increasing in the level of this chemoattractant in pregnant women with acute toxoplasmosis infections. The present study compatible with the findings of a study Mieliauskaite *et al.* [32] have registered a significant rise in the level of TGF- 1 in RA patients. The results of the present study disagree with the lack of significant differences between the patients of RA and the healthy inTGF- 1 level [33].

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