Detection of *Toxoplasma* Antibodies and TNF-α in Rheumatoid Arthritis Patients Treated with Methotrexate

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Abstract

This study was conducted to evaluate the prevalence rate of toxoplasmosis among 294 rheumatoid arthritis (RA) patients treated with methotrexate (MTX), 50 RA patients without treatment and 50 samples as healthy control. Blood samples were collected and the presence of *T.gondi*i IgG and IgM antibodies was determined by using Enzyme linked immunosorbent assay (ELISA). Tumor necrosis factor alpha (TNF-α) was also estimated in serum of all subjects by using ELISA method too. The seroprevalence of toxoplasmosis IgM and IgG in RA+MTX was 60(20.408%), and 98(33.33%), in RA patients 4(8%), and 18(36%) while, it was 2(24%), 6(12%) in healthy group. Tumor necrosis factor alpha (TNF-α) was also estimated in serum of all subjects by using ELISA method too. The mean levels of TNF-α in seropositive anti-Toxoplasma IgM and IgG of RA+MTX patients were 3.781 pg/ml ± 0.571) and (36.98 pg/ml ± 0.58), in RA patients (25.404 pg/ml ± 1.748) and (40.12 pg/ml ± 1.7) while, they were (5.04 pg/ml ± 0.643) and (10.7 pg/ml ± 1.7) in healthy group. The results showed significant difference ($P<0.05$) was found between treated and untreated patients.

Keywords: Rheumatoid Arthritis, methotrexate, Tumor necrosis factor alpha.

التحري عن الاجسام المضادة للمقوسات الكونيدية و عامل التنخر الورمي في مرضى التهاب المفاصل الرثياني المعالجين بالميثوتريكسيت

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الخلاصة

أجريت هذه الدراسة لتقييم معدل انتشار داء المقوسات الكونيدية في294 مريض بمرض التهاب المفاصل الرثياني المعالجين بالميثوتركسيت, و50 مريض بمرض المقاصل التهاب الرثياني غير معالجين و50 عينة كعينات سيطرة. تم جمع العينات وتحديد وجود الأجسام المضادة لطفيلي المقوسات الكونيدية من النوع (ج) و (م) باستخدام تقنية الاليزا. كذلك تم قياس عامل التنخر الورمي بتقنية الاليزا أيضا. وكانت الأجسام المضادة لطفيلي المقوسات الكونيدية من النوع (ج) و

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(م) في مجموعة التهاب المفاصل الرثياني المعالجين بالميثوتركسيت (20.408%)60 و (33.33%)98, ولمرضى التهاب المفاصل الرثياني غير المعالجين (8%)4 و (63%)18 أما في مجموعة السيطرة (24%)2 و(12%)6. وكان معدل مستوى عامل التنخر الورمي في الأشخاص المصابين بطفيلي المقوسات الكونيدية من النوع (ج) و (م) في مجموعة التهاب المفاصل الرثياني المعالجين بالميثوتركسيت (3.871pg/ml±0.571) و (36.98pg/ml±0.58), ولمرضى التهاب المفاصل الرثياني غير المعالجين (25.404 pg/ml ±1.748), (40.12 pg/ml ± 1.7) وفي مجموعة السيطرة (5.04 pg/ml ± 0.643), و (10.7 pg/ml ± 1.7). أوضحت النتائج وجود فروق معنوية (P<0.05) بين المرضى المعالجين وغير المعالجين .

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease .It is a chronic multisystem disease of unknown cause. Although there are a variety of systemic manifestations, the characteristic feature of established RA is persistent inflammatory synovitis, usually involving peripheral joints in a symmetric distribution [1]. The potential of the synovial inflammation to cause cartilage damage, bone erosions and subsequent changes in joint integrity is the hallmark of the disease. Despite its destructive potential, the course of RA can be quite variable. Some patients may experience only a mild oligoarticular illness of brief duration with minimal joint damage, but most will have a relentless progressive polyarthritis with marked functional impairment. The prevalence of RA is ~ 0.8% of the population (range between 0.3–2.1percent). Women are affected approximately three times more often than men [2]. The release of specific cytokines into the systemic circulation had been observed in a variety of inflammatory disease, including RA. Their concentration levels usually reflect disease severity and prognosis [3]. Interlukin-1 and tumor necrosis factor- alpha (TNF-α) have been characterized as the major pro-inflammatory cytokines in the inflamed joint in RA. They have overlapping actions including local inflammation, enhancing adhesive properties of the inflammatory cells, causing angiogenesis, and bone resorption [4].

The treatment of RA has undergone somewhat of a revolution over the last decade, with a strong consensus emerging in favor of early aggressive therapy [5]. Now there is evidence that early treatment of the disease has a beneficial impact on treatment outcome. The goals to be achieved in managing RA are prevention or control of joint damage, prevention of loss of function, and reduction of pain [6]. Methotrexate (MTX) is a chemotherapy used in low doses (5–25 / mg/week) for RA and other inflammatory conditions [7]. MTX inhibits production of proinflammatory cytokines including TNF-α, Interferon -gamma, which are important in the inflammatory process in RA [8]. Low-dose of MTX can inhibit immunoglobulin synthesis and neutrophil chemotaxis and can cause bone marrow suppression [9]. However, concern remains regarding the vulnerability to infection and increased severity of infections in patients taking low-dose MTX [10]. Low-dose weekly of MTX is associated with opportunistic infections like tuberculosis, histoplasmosis and toxoplasmosis as early as a few weeks to several years after starting therapy [11]. Toxoplasmosis caused by the ubiquitous obligatory intracellular coccidian protozoan organism, *Toxoplasma gondii* [12]. *Toxoplasma gondii* is among the most prevalent parasites in the global human population, with around one third of the population being infected [13]. *T. gondii* is an opportunistic parasite infects the immune compromised hosts [14].This study aimed to determine the prevalence of toxoplasmosis infections which is asymptomatic in immunocompetant persons, but in individuals who are immunocompromised, the parasites can become widely disseminated.

Materials and methods

Subject

From September 2013 till the end of February 2014, 294 sera from RA patients treated with MTX for at least two months ago, as well as 50 healthy individuals and 50 RA patients first investigation (untreated). Samples were collected from Department of Rheumatology, Baghdad Teaching Hospital and Al- Ulwia Hospital in Baghdad, in addition to outpatient

clinics. The patients age ranged between 20-80 years. Five ml of blood was collected from each subject, the tubes were centrifuged at 2,000 rpm for 5 min and the sera were liquats in several vials and kept at -20 C.

Serology

Enzyme linked Immunosorbent Assay (ELISA) kit (BIOChek, Inc, Foster City, CA) was used for detection specific anti-*Toxoplasma* IgG and IgM antibodies in the sera of all subjects according to the manufactures instructions. To detrmine Tumor Necrosis Factor (TNF) –α [Human Enzyme Immunoassay Demeditec kit (EN ISO 9001)] was used in all subjects sera according to the manufactures instructions [15].

Statistical Analysis

Statistical analysis system program- SAS (2004) was used for data analysis. Person Chi-square -χ^2 test, and mean ± SE, ANOVA Table by using computer program IBM SPSS version [16]. P value <0.05 was considered statistically significant.

Results and discussion

Blood samples were analyzed for specific anti-*T.gondii* IgG and IgM antibodies using ELISA tests to estimate the actual percentage of toxoplasmosis in RA patients treated with MTX. The presence of acute toxoplasmosis characterized by the presence of positive IgM antibodies, the current study was shown that patients whom treated with MTX have 60(20.408%) seropositive anti-*Toxoplasma* IgM antibodies, 234(79.592%) seronegative anti-*Toxoplasma* IgM antibodies comparing with untreated RA patients and healthy control who have 4(8%) RA seropositive anti-*Toxoplasma* IgM antibodies, 46(92%) seronegative anti-*Toxoplasma* IgM antibodies and 2(4%) seropositive anti-*Toxoplasma* IgM antibodies, 48(96%) seronegative anti-*Toxoplasma* IgM antibodies, respectively as sown in table 1. Results were calculated by drawing a standard curve. Plotting on the horizontal axis the TNF-α concentration of the standards, and on the vertical axis the corresponding absorbance.

The average absorbance for each sample was located on the vertical axis and the corresponding TNF-α concentration on the horizontal axis was read as shown in figure 1. The statistical analysis showed significant differences (P<0.05) between studied groups.

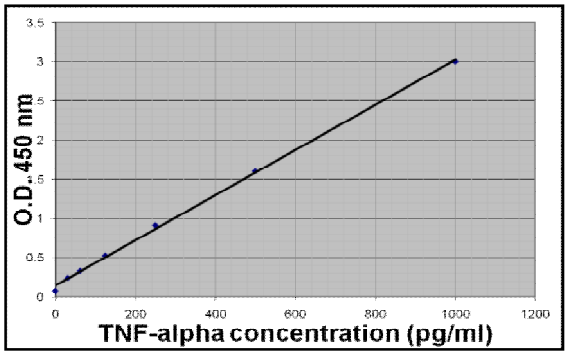

Figure 1-Standard curve of total TNF-α concentration

Table 1-The percentage distribution of RA patients infected with toxoplasmosis diagnosed by ELISA test (IgM).

Test / Subject	ELISA-IgM		Total
	Positive	Negative	
RA+MTX	60(20.408%)	234(79.592%)	294
RA	4(8%)	46(92%)	50
Healthy	2(24%)	48(96%)	50
Person Chi-square - χ^2	11.217*		* P< 0.05

***P< 0.05 significant differences**

Chronic toxoplasmosis characterized by the presence of positive IgG antibodies only, chronic infection in RA patients treated with MTX recorded 98(33.33%) seropositive, and 196(66.67%) seronegative anti-*Toxoplasma* IgG antibodies comparing with 18(36%) seropositive, and 32(64%) seronegative anti-*Toxoplasma* IgG antibodies in untreated RA patients and 6(12%) seropositive, and 44(88%) seronegative anti-*Toxoplasma* IgG, in healthy control as shown in table 2. The statistical analysis showed significant differences between them ($p<0.05$).

Table 2-The percentage distribution of RA patients infected with toxoplasmosis diagnosed by ELISA test (IgG).

Test Subject	ELISA-IgG		Total
	Positive	Negative	
RA+MTX	98(33.33%)	196(66.67%)	294
RA	18(36%)	32(64%)	50
Healthy	6(12%)	44(88%)	50
Person Chi-square - χ^2	5.934*		*P< 0.05

***P< 0.05 significant differences**

Walle *et al.,* [17] has been use ELISA for toxoplasmosis diagnosis in HIV patients showed that out of 103 positive results 11(10.7%) gave IgM positive and 90(87.4%)gave IgG positive, our results were higher in IgM positivity but lower in IgG titer. The treated RA patients with MTX were highly infected with *T. gondii* comparing with untreated RA patients and healthy subjects. Our results were higher than [18] who recorded that acute toxoplasmosis in RA patients was 5%, while chronic infection was 42 %. RA disease causes an increase in the body's immune response, and hence the parasite cannot make an acute infection, so the parasite remains in the bradizoite form. The current study clarified that the incidence of chronic was higher than the acute infection while the incidence of acute infection in RA+MTX group was higher than chronic infection due to treatment action on immune response. *T. gondii* is an opportunistic parasite infects the immune compromised hosts [19].

Levels of TNF-α during acute infection with toxoplasmosis were lower than levels in other studied groups [20], while TNF-α in untreated RA patients were significantly elevated than other studied groups, these data confirmed the previous studies which donated that TNF-α involved in the aetiopathogenesis of RA [21].Based on these findings, blockers of TNF-α or its receptors have been approved to be effective treatment in the case of these autoimmune disease [22].

The current study was shown that TNF-α level in chronic infection was higher than acute infection as sown in table (3). The statistical analysis was shown significant differences ($P<0.05$) between studied groups. levels of TNF-α during acute infection with toxoplasmosis were lower than levels in other studied groups [20], while TNF-α in untreated RA patients were significantly elevated than other studied groups, these data confirmed the previous studies which donated that TNF-α involved in the aetiopathogenesis of RA [21].Based on these findings, blockers of TNF-α or its receptors have been approved to be effective treatment in the case of these autoimmune disease [22].

Table 3-Means levels of serum TNF-α in *Toxoplasma*-IgM & IgG among studied groups.

Studied groups	Mean± SEM (pg/ml)			
	No	***Toxo* IgM**	**No**	***Toxo* IgG**
RA+MTX	60	3.781 ± 0.571*	98	36.98 ± 0.58*
RA	4	25.404 ± 1.748	18	40.12 ± 1.7
Healthy	2	5.04 ± 0.643	6	10.7 ± 1.7
Statistical analysis ANOVA			P< 0.05	

***P< 0.05 significant differences**

In this study acute and chronic infections of *T. gondii* found, that the RA patients whom were treated with MTX become immune compromised, hence they will be easily to infect or reactivate infection with *T. gondii*. MTX will block the production of TNF-α, and because TNF-α mediates the immune response by increasing the transport of white blood cells to sites of inflammation, and through additional molecular mechanisms which initiate and amplify inflammation. Inhibition of its action by MTX reduces the inflammatory response which is especially useful for treating autoimmune disease, hence *T. gondii* parasite will be easily to infect human [23].

TNF-α concentration in acute toxoplasmosis was lower than its concentration in chronic infections, that's means the TNF-α has very important role in the immune response against *T. gondii* infection and other pathogenic infection. Tachyzoite the active form of *T. gondii* infection results in suppressed TNF-α cytokine production in infection by this parasite [20]. The results presented here suggest that *Toxoplasma* suppressed proinflammatory cytokine production (TNF-α) in order to initiate the infection and MTX also help in blocking the production of TNF-α [24], so RA patients treated with MTX will under the risk of toxoplasmosis infection.

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