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Role of Interleukin 33 During Infection with Toxoplasmosis in Rheumatoid Arthritis Patients

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Abstract

Toxoplasma gondii is avery common obligate intercellular parasite with highest infection rate among worlds populations. The Rheumatoid arthritis (RA) considered as an autoimmune disease characterized by loss immunological tolerance to self-antigens. Present study Aims to detection the seroprevalence of toxoplasmosis among RA patients and healthy control and evaluate concentrations of IL-33 to understand their role during infection. Seropositive cases of IgG was 36.7% percentage in RA patients with highest value of IgG 0.395 IU/ml while control group was 100% seronegative. Three cases only were seropositive to IgM with 1.17% and their mean 0.54 IU/ml.

IL-33 levels in RA patients with toxoplasmosis was highest 187.74± 33.24 pg/ml when compared with RA patients 107.88±18.41 pg/ml and control group 55.59±25.75 pg/ml ,there was a significant differences in comparison between studied groups.

Key words:- *Toxoplasmosis , Rheumatoid arthritis, IgG , IgM , IL-33.*

Introduction

Toxoplasmosis is a common an obligate intracellular protozoan parasite zoonotic disease infectious by *Toxoplasma gondii ,* classified as a Coccidia and phylum Apicomplexa [(1)]. All or most marsupials and mammals can serve as intermediate hosts, while several Felidae, enrolled domesticated cats, are the final hosts for *T. gondii* [(2)], Human infection generally happened by uptake raw uncooked meat which have cysts (or possibly tachyzoites). animals, including herbivores can be infected by eating vegetables and plants contaminated with oocysts. However, infections can be transmitted through placenta from mother who suffered from infection during pregnancy [(3)]. Infection leading to encephalitis in the fetus ,cerebral calcification and mental retardation or psychomotor , *T. gondii* may be transmitted by transplanted organs or transfused blo od[(4)].

The primary or secondary infections with *Toxoplasma gondii* can mimicry immune response and make change in the immune response to generate autoimmune disease including autoimmune thyroid diseases, inflammatory bowel disease ,systemic sclerosis and rheumatoid arthritis [(5,6)]. Rheumatoid arthritis is an autoimmune disease described by inflammatory polyarthritis of small and big joints, can cause major disability and uneasiness [(7)]. That affects approximately 0.5% to 1% among adults a worldwide [(8-9)]. the pathogenesis of this infection is multifactorial including genetic and ecological factors [(10)]. it is identified by peripheral joints progressive inflammatory synovitis , cartilage damage, corrosions of bone and production of autoantibodies like rheumatoid factor and antibodies of the anti–citrullinated protein [(10)].

The present study was aimed to evaluate levels of interlukine 33 (IL-33) in patients with rheumatoid arthritis and infected toxoplasmosis infected which diagnoses by anti-*Toxoplasma* antibodies including IgG and IgM seroprevalence

Material and method

Study subjects

Study was implemented as A case-control study to identify the IL-33 role in the prevalence of *T. gondii* seropositivity in rheumatoid arthritis patients . samples were collected from patients attending to the department

of Rheumatology in Baghdad and Al-Kindi Teaching Hospitals in Baghdad province, in addition to outpatients clinics according to ethical approval of environmental and health ministry of Iraq . During March to July 2018, samples included 256 of rheumatoid arthritis patients that treated with methotrexate (207 females and 49 males) their age ranged from (15 – 65) years with a mean (40.77± 1.5), and healthy individual consisting of 50 person of healthy people (16 females and 34 males) did not suffer from any pain in the joints and didn't have a satisfactory history of any autoimmune diseases.

serological testing

about five ml of venous blood were collected from patients and control groups in gel tube , its left at room temperature for 15 minutes then its centrifuged for 5 min at 3000×g . serum were collected in eppendorf tubes and stored at - 20 .

The clinical diagnosis of rheumatoid arthritis implemented specialist physician in addition of clinical examination and several laboratory tests including Rheumatoid Factor (RF) (Biosysteme , Spain) and C- Reactive Protein test (CRP) (Spinract ,Spain). The *T. gondii* antibodies including IgG and IgM were analyzed using ELISA kits (Bioactiva, Germany),

Interleukin level was determined by a commercially available human ELISA kits for IL-33 (Peprotech , USA). all tests were implemented according to manufactures instructions.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to data analysis. The data was shown 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 0.01 and 0.05 to assess the differences between the study groups .

Results and Discussion

The results of present study were shown that 36.71% were seropositive cases of IgG in 94 patients with RA compared to 162 seronegative cases at 63.2%, while the control group was 100% seronegative. High significant differences recorded between studied groups ($p \leq 0.001$) (table 1) .the infections of Parasitic may encourage immunomodulatory variable impacts and control of autoimmune disease [11]. Perhaps the most interesting association of *T. gondii* with other diseases is with rheumatoid arthritis. In humans, *T. gondii* infection may cause a symmetrical polyarthritis of the minor joints of hands, fingers, wrists and knees [12], as well as *T. gondii* may be implicated in autoimmune disease through acting as a ligand for toll-like receptors (TLRs) [11].

Table (1): the percentages of *T. gondii* infection according to **Toxo IgG IU/ml ELISA assays in studied gr oups.**

Parameter	results	Rheumatoid Arthritis		Control P-value		
		No.	%	No.	%	%
IgG Antibody	Positive	94	36.71	0	0.00	0.0001 **
	Negative	162	63.2	50	100	
Total		**256**		**50**		
P-value		0.0001 **				

Table (2) shows that the level of IgM was elevated in group of rheumatoid arthritis patients infected with toxoplasmosis compared with other groups with highly significant differences .

Table (2): IgM levels estimated by IU/ml for studied groups.

Groups	No.	Mean	Std. Dev.	Std. Error	LSD	P-value
RA with toxoplasmosis	3	0.540	0.062	0.029	0.194 **	0.00061
R.A patients	253	0.0810	0.022	0.003		
Control	50	0.063	0.018	0.006		

In the current study, the relation between toxoplasmosis and RA in Iraqi patients was been recognized by the detection of antibodies IgG and IgM in sera of the studied groups by ELISA test , ELISA is an precise serological tool for the diagnosis of toxoplasmosis dependent on Ag - Ab interaction. The reaction can be evaluated objectively by quantization of the color that advanced by an ELISA reader. This technique is economical and also suited for analyzing large number of samples at the same time[13]. The present study is agree with Kuba *et al*.[14] which found that the seroprevalence of *T. gondii* IgG and IgM antibody were 33.33% and 20.40% respectively in RA patients treating with methotrexate, and 36.00% and 8.00% in RA patients without treatment, while it was 12.00% and 24.00% in healthy control. Salman and Mohammed [15] found in Kirkuk city that the percentage seroprevalence of toxoplasmosis of IgG and IgM were (47.54% -6.55%) respectively in RA patients. El-Henawy *et al*.[16] showed that Anti-Toxoplasma IgG antibodies were positive in (76.7%) of patients with RA versus controls (48.3%), they suggested that higher seroprevalence of anti-Toxoplasma IgG antibodies in RA group refer to correlation between latent *Toxoplasma* infection and RA. Conversely, other study, show no significant difference of IgG levels which found between RA group and controls and *T. gondii* infection might be an incidental result [17].

The elevated seroprevalence of anti-*Toxoplasma* IgG antibodies in RA group may be expected as a result of diverse cooking methods and oocysts ability to live in different weathers in addition of a close association with cats (18). An increased risk of *T. gondii* infection in patients suffered from rheumatic diseases can be share in to changes in innate and adaptive immune responses [19]. The Patients of RA were located to be highly vulnerable to *T. gondii* infection - particularly throughout periods of immunosuppression that followed treatment with TNF-α inhibitors [20].

Table (3) illustrated that all samples of rheumatoid arthritis have positive results for the test index RF, CRP ,except for the control group, which showed the complete passivity of both tests and with highly significant differences p≤0.0001.

Table (3): index of the Rheumatic Factor and C- Reactive Protein test of the study groups.

Diagnosis	Response	Patients		Rheumatoid Arthritis		Control		p-value
		No.	%	No.	%	No.	%	
RF	Ve+	97	100	159	100	0	0.00	0.0001 **
	Ve-	0	0.00	0	0.00	50	100	
CRP	Ve+	97	100	159	100	0	0.00	0.0001 **
	Ve-	0	0.00	0	0.00	50	100	
Total		97		159		50		

of the elevation of rheumatoid factor (RF) titers in serum and synovial fluid is a characteristic feature of RA and perhaps implicated in pathogenesis of this disease [21]. Rheumatoid factors are autoantibodies with specificity for the Fc portion of IgG and may be formed in multiple iso-types [22]. The results of the test rheumatoid factor were similar with El-Henawy *et al*.[16] who found the mean of patients of rheumatoid arthritis with toxoplasmosis of 46 patients to be 74.01±8.62 (IU/mL) , while the mean of patients without toxoplasmosis was 64.56±3.09 (IU/mL) represented 14 patients.

There is another possible marker for increasing the danger of rheumatoid arthritis, is a C-reactive protein (CRP), which one of the acute phase reactants that increase in inflammation response [23]. The results of the current study were agree with Salih [24] which recorded in a study that women in the Kurdistan region suffering from rheumatoid arthritis and all samples were positive for the RF and CRP tests. These autoantibodies are linked with *T. gondii* especially when they are of high rates [25].

Table (4) shows that the level of IL-33 in the group of RA cases infected with toxoplasmosis was high levels with highly significant differences than other groups.

Table (4): Levels of IL-33 estimated by pg/ml for study groups.

Groups	Number	Mean	Standard deviation	Standard errors	P-value	LSD
RA patients with toxoplasmosis	50	187.74	33.24	5.01	0.0001	39.427 **
RA Patients	50	107.889	18.41	4.11		
Control	50	55.59	25.75	5.75		

Table (5) revealed the comparisons of IL-33 levels between the studied groups.

Table (5): Multiple comparisons of IL-33 for potential couples between study groups.

Indicator	Group 1	Group 2	mean difference	P-value	Sig.
IL-33 Concentration (Pg/ml)	RA patients with toxoplasmosis	RA patients	79.851	0.0075	HS
		Control	132.15	0.0002	HS
	RA patients	Control	52.299	0.0093	HS

The Interleukin-33 (IL-33) is reported as one of cytokine of IL-1 family, which can be induced cytokine syntheses and inflammatory responses mediated through its receptor ST2. It is expressed in several tissues and cells like liver, lung, central nervous system also epithelial, endothelial, smooth muscle, macrophages and fibroblasts cells [26]. The pathway of IL-33/ST2 have major role in defense of host and in immune organization in inflammatory and infectious diseases [27]. IL-33 have capability to elevated the Th2 immune response and lowering the production of Th1 cytokines [28] also it plays major role in innate and adaptive immunity [29]. The gene of IL-33 is located in chromosome 9 [30]. Mature IL-33 mediate its biologic effects via T1/ST2 binding by activating NF-κB and MAP kinase [31] .The present study is agree with AL-Shammaa [32] which showed a highly significant increase IL-33 in concentrations in sera of toxoplasmosis female

of Iraqi patients compared with the healthy control. This finding may indicate that toxoplasmosis might influence cytokine production in these patients [33].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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