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The Impact of *Toxoplasma Gondii* Infection on the Level of Shla-G and its Receptor in Iraqi Patients Infected with Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis is an autoimmune disease characterized by insistent inflammation of joints which will developed to damage of cartilage and effect on the life quality and mortality. 196 subjects were included in this study, divided into (RA+ve toxo-ve , RA+ve toxo +ve, Toxo + ve RA -ve, and RA-ve toxo -ve groups . The C-reactive was measured, Rheumatoid Factor, and anti- Toxoplasma gondii (IgM and IgG), and Human Major Histocompatibility Complex class 1G, and Human Leukocyte Immunoglobulin Like Receptors sub family B member 4 by ELISA method using (My bio source kits/USA).The result showed that the percentage of patients who had positive this study included only patients with positive results for both tests CRP and RF which was just (64%), and The results showed that treated RA patients without Toxoplasma had the highest significant (P≤0.01) increase level of sHLA-G in comparison to RA untreated patients, also treated RA co infected patients with Toxoplasma showed high significant increase (P≤ 0.01) of sHLA-G in comparison to co infected untreated, while the level of sHLA-G in patients with toxoplasmosis only significantly (P≤0.01) increased in comparison to RA-ve toxo -ve, whilst that treated RA patients without Toxoplasma had the highest significan t(P≤0.01) increase level of LILRB4 in comparison to RA untreated patients , while, treated RA patients with Toxoplasma showed high significant increase (P≤0.01) of LILRB4 in comparison to RA untreated patient, Also the results of the level of LILRB4 in patients with toxoplasmosis only showed significantly (P≤0.01) decreased in comparison to RA-ve toxoplasmosis -ve

.**Key words:** Rheumatoid arthritis, *Toxoplasma gondii*, HLA-G, LILRB-4.

Introduction

Rheumatoid arthritis (RA), considered as one the most well-known chronic type of autoimmune arthritis (Merola et al., 2018), this autoimmune disease characterized by insistent inflammation of joints which will developed to damage of cartilage

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(Calabresi et al., 2018). This disease effect on the life quality and mortality (because the RA has complication such as elevated risk of cardiovascular events (Chimenti et al., 2015; NHS., 2019), expected threats can be reduced cognitive function in the brain, fibrotic disease in the lungs, osteoporosis, and a high danger of developing cancers (Guo et al .,2018). Toxoplasmosis is a zoonotic disease caused by the protozoan parasite Toxoplasma gondii, the genus Toxoplasma while the species gondii, which is infect the intestinal of cats, this parasite have been first discovered during 1908 by Manceaux and Nicolle (Ibrahim, 2017). Additionally this parasite has an importance as it infect two hosts humans and animals (cats as definitive host and other worm blooded mammals act as intermediate host) (Tucker and Abigail ,2016). The set of histocompatibility genes responsible of encoding different types of proteins that works as a regulator of host-pathogen interactions. Unarguably, the most important molecules in this types of proteins are the HLA, as it is a glycoproteins located in the cell-surface works by binding peptide fragments of proteins which are either have been synthesized within the cell (class Ia HLA) or that have been ingested by the cell and proteolytically processed (class II HLA). Although antigens that is occur after the processing of pathogens are presenting on the classical HLA molecules and this function as protector of cells, there is a subset of HLA genes that account for encoding non-classical class Ib molecules that play a role on the modulation of immune response (Persson et al., 2017). The LILRs, also known as CD85 and ILTs, are a family of type I transmembrane glycoproteins with extracellular Ig-like domains that bind ligands and ITAMs or ITIMs. LILRBs possess 2 or 4 Ig-like domains and a long cytoplasmic tail with ITIMs. Contrariwise, LILRAs have 2 or 4 Ig-similar domains that is has short cytoplasmic tails and associate with an FcγR chain containing ITAMs. The soluble forms of LILRs without transmembrane and cytoplasmic regions are generated by alternative splicing, thereby serving as decoy regulators. In contrast, although no receptor ligation studies have been performed for LILRB4 on antigen presenting cells, at least some of its inhibitory effects may result from reverse signalling through its T cell ligand, as soluble forms of LILRB4 have been shown to suppress T cell responses. LILRB4 ligation was therefore performed to determine the phenotypic effects of receptor engagement on antigen presenting cell phenotype (Vlad et al., 2008).

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Materials and Methods

Two hundred samples are included in this study collected from Department of Rheumatology, Baghdad Teaching Hospital ,and Al- Numan Teaching Hospital in Baghdad, in addition to outpatient clinics , Baghdad , Iraq, and the collection was during the period from September 2019 till the end of February 2020, both genders suspected with RA infection age ranged (20-80 years), and 100 samples were taken from healthy donors to detect of IgM and IgG positive group Toxoplasma antibodies, while the negative considered as a control. The C-reactive was measured (CRP), Rheumatoid Factor (RF) using (spinreact/Spain), and anti- Toxoplasma gondii (IgM and IgG) the determination of antibodies occurred by enzyme linked immunosorbent assay (ELISA) method using (BioCheck kit/USA), while Human Major Histocompatibility Complex class 1G, and Human Leukocyte Immunoglobulin Like Receptors sub family B member 4 by ELISA method using (My biosource kit/USA).

Results and Discussion

This study involved 200 patients who were suspected of having RA disease and were all given RA screening tests (CRP and RF). The results revealed that the percentage of patients with positive CRP (90 %) on the other hand, positive RF were (70 %). Only patients with positive outcomes for both tests were included in this study which was only (64%) patients, as seen in table(1).

Table (1): Samples distribution according to CRP and RF test.

Total samples 200			
Test	**CRP**	**RF**	**CRP and RF**
percentage of $^{+ve}$ samples	**90%**	**70%**	**64%**

Positive result for CRP concentration > 6 mg / L , Positive result for RF concentration > 30 IU/ml.

All the 96 RA patients and 100 healthy donors were subjected to ELISA Toxo IgM and IgG tests. The result obtained that no acute toxoplasmosis (-ve IgM) was detected in RA patients while 40/96 (41.7%) with chronic toxoplasmosis (IgG +ve). However, out of 100 samples from healthy donors, the result showed that no IgM +ve for toxoplasmosis, while, 35 (35%) were positive for IgG only. Thus those patients considered positive for toxoplasmosis and the other 65 healthy donors were control group. As seen in table (2).

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Table (2): The percentage of antitoxoplasma antibodies IgM and IgG in the current samples.

Cases	No. of cases	IgM +ve	IgG +ve	IgM-ve , IgG-ve
RA	**96**	**None**	**40 (41.7 %)**	**56 (58.3 %)**
Healthy donors	**100**	**None**	35 (35 %)	65 (65 %)

The current results showed that treated RA patients without Toxoplasma had the highest significant (P≤0.01) (10.72 ± 0.28) increase level of sHLA-G in comparison to RA untreated patients (5.81 ± 0.43).

However, treated RA co infected patients with *Toxoplasma* showed high significant increase (P≤ 0.01) of sHLA-G (5.21 ± 0.21) in comparison to co infected untreated (3.85 ± 0.35).

Additionally when looking to the result of level of sHLA-G in patients with toxoplasmosis only, the result obtained that its level significantly (P≤0.01) increased (4.77 ± 0.24) in comparison to RA-ve toxo -ve (2.49 ± 0.08).As seen in table (3).

Table (3): Comparison between difference groups in HLA-G.

Group		No	Mean ± SE of HLA-G
RA+ve toxo-ve	Treated	40	10.72 ± 0.28 [a]
	Un treated	16	5.81 ± 0.43 [b]
RA+ve toxo +ve	Treated	28	5.21 ± 0.21 [bc]
	Un treated	12	3.85 ± 0.35 [d]
Toxo + ve RA -ve		35	4.77 ± 0.24 [c]
RA-ve toxo -ve (Control)		65	2.49 ± 0.08 [e]
LSD value		--	0.739 **
P-value		--	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).			

The current results showed that treated RA patients without *Toxoplasma* had the highest significan t(P≤0.01) (4.19 ± 0.14) increase level of LILRB4 in comparison to RA untreated patients (2.81 ± 0.11).

While, treated RA patients with Toxoplasma showed high significant increase (P≤0.01) of LILRB4 (2.41 ± 0.06) in comparison to RA untreated patients

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(1.90 ± 0.11).

Also the results of the level of LILRB4 in patients with toxoplasmosis only showed significantly (P≤0.01) decreased (0.80 ± 0.04) in comparison to RA-ve toxoplasmosis -ve (1.49 ± 0.06). As seen in table (4).

Table (4): Comparison between difference groups in LILRB4.

Group		No	Mean ± SE of LILRB4
RA+ve toxo-ve	Treated	40	4.19 ± 0.14 a
	Un treated	16	2.81 ± 0.11 b
RA+ve toxo +ve	Treated	28	2.41 ± 0.06 c
	Un treated	12	1.90 ± 0.11 d
Toxo + ve RA -ve		35	0.80 ± 0.04 f
RA-ve toxo -ve(Control)		65	1.49 ± 0.06 e
LSD value		--	0.316 **
P-value		--	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).			

The results of this study showed, serum sHLA - G protein concentration is significantly lower in untreated RA patients this result was agreed with previous result by Sonoda *et al.*, (1990) the decreased sHLA –G can result to a chronic activation of inflammatory cells and this inflammation INCREASE the severity of the disease (Rizzo et al., 2014). Other study by, LeMaoult et al. (2003) find a higher concentration of sHLA-G molecules at the specific inflammation site of the synovia in RA patients. Rizzo *et al.* (2014). Interestingly, numourus studies find a positive correlation between the disease severity and the low sHLA-G and membrane HLA –G expression, while there was an up modulation of sHLA-G was evident after three months of DMRADs therapy, this result came in line with the present result that showed there was a significant increase in sHLA-G level in treated patients in comparison with untreated one. These result proved that T and NK cells activation are not adequately down regulated by sHLA-G molecules as sHLA-G serum level significantly decreased in patients in RA and positively correlated with parameters of disease activity (Verbruggen *et al.*, 2006). However, Ismail et al.(2019) showed how the infection T.gondii associated with many autoimmune diseases and testing for Toxoplasma infection in RA patients to prevent any further complications. This association was present also in the current study that showed about (41.7%) of RA patient co infected with Toxoplasma gondii parasite and however agreed with El-

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henawy *et al.*(2017) who detect higher anti- Toxoplasma IgGs in Egyptian RA patient compared to controls and with El-sayed et al.(2016) , while the study by Ismaile *et al.* (2019) failed to prove the exact role of Toxoplasma whether a trigger of autoimmunity in autoimmune diseases or an effect of immunosuppression and recommend to increase sample size and further large scale studies to. Infection with parasite could trigger the chronic disease development; on the other hand, chronic disease controlled by immunosuppression drugs may increase susceptibility of patient to infections including toxoplasmosis (Flegr et al., 2014).

The result showed that patients with RA disease without toxoplasmosis characterized by high significant increase expression of LILRB4 receptor in comparison to untreated one. This result considered rationality especially when RA patients respond to treatment and the inflammation impact were dampen and subsided.

Sever infections with Toxoplasma are well-known to occur in RA patients treated with immunosuppressive drugs (Ali and Dilaver.,2019).This result coordinate with current study.To discuss this finding, previous researchers explained that LILRB4 is predominantly expressed on macrophage and DC and its an inhibitory receptor that specific to immune tolerance Svensson-Arvelund *et al.*,(2015) and Li *et al.*, (2017) observed the presence of high level of LILRB4 on UN infected mouse decidual macrophage but low level of this receptor with abnormal pregnancy outcomes mediated by *T.gondii* infection. In spite of the increased level of sHLA-G in all patients groups, patients with toxoplasmosis only, reveled down-regulation of LILRB4 expression, which means that no enough receptor (LILRB4) for this amount of sHLA-G in patients srea. Leukocyte immunoglobulin-like receptor (LILRB4) is a type of inhibitory receptor that plays a major role in immune checkpoint pathways, and participate in achieving balance between activating and inhibitory actions to ensure immune responses to pathogen .As immune checkpoints are of great significance in autoimmune diseases, LILRB4 is a target for treating autoimmune diseases. LILRB4 is associated with many kinds of immune diseases, in addition, LILRB4 plays an effective role in inflammatory diseases (Deng *et al.*, 2021).

Later studies found that the expression levels of functional LILRB4 molecules in the membrane, arginine metabolizing enzymes and related cytokines were abnormal in Toxoplasma gondii infection models, demonstrating that Toxoplasma infection can down regulate LILRB4 in decidual macrophages (Li et al., 2017).

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