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Original paper

The effect of *Toxoplasma gondii* on some critical immunological markers in rheumatoid arthritis Iraqi patients

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ABSTRACT. Toxoplasmosis caused by *Toxoplasma gondii* (*T. gondii*) affects one-third of the world human population. One of immune evasion strategy in the host-parasite interplay is HLA-G level alteration. HLA-G known as a special proteins (non-classical HLA class I) molecules which can suppress the immune system and its capability of modulating natural killer cell (NK) function such as cytotoxicity and cytokine production through NK cell receptors, specially KIR2DL4 receptor. KIR2DL4 a member of KIR family, featured with both inhibitory and activating functional structure, has been described as the specific receptor for HLA-G. So, this study aimed to investigate the alteration in sHLA-G and its receptor levels could be impressed by present of *Toxoplasma* and rheumatoid as well as the seroprevalence of *Toxoplasma gondii* antibodies in Iraqi patients with rheumatoid arthritis (RA) was investigated. The prevalence of anti-*T. gondii* IgG was significantly higher in arthritic patients (50%) compared with (41.6%) in healthy controls. No positive anti-*T. gondii* IgM was detected. The results showed that treated RA patients without *Toxoplasma* had the highest significant ($P \leq 0.01$) increase level of sHLA-G in comparison to RA untreated patients, also treated RA patients without *Toxoplasma* showed high significant increase ($P \leq 0.01$) of sHLA-G in comparison to untreated RA patients, while the level of sHLA-G in patients with toxoplasmosis only significantly ($P \leq 0.01$) increased in comparison to control, whilst that treated RA patients without *Toxoplasma* had the highest significant ($P \leq 0.01$) increase level of KIR2DL4 in comparison to RA untreated patients, while, treated RA patients with *Toxoplasma* showed high significant increase ($P \leq 0.01$) of KIR2DL4 in comparison to RA untreated patient. Also, the results of the level of KIR2SL4 in patients with toxoplasmosis only showed significantly ($P \leq 0.01$) decreased in comparison to control. The present study describes the change in HLA-G and KIR2DL4 levels in present of *Toxoplasma* and RA.

Keywords: *T. gondii*, rheumatoid arthritis, HLA-G

Introduction

Toxoplasmosis is a widely distributed zoonotic infection caused by the obligate intracellular apicomplexan parasite *Toxoplasma gondii*. Humans or animals can acquire *T. gondii* infection postnatally by ingestion of undercooked or raw meat from infected animals, or ingestion of food or water contaminated with oocysts excreted by infected felids [1].

Felids are considered as the only definitive hosts of *T. gondii* playing a crucial role in the transmission of the parasite [2]. This parasite in immunocompetent people is generally without side effects, however nowadays its accepted that the disease can be a risk factor for an assortment of immune system sickness including rheumatoid arthritis (RA) [3].

Rheumatoid arthritis is a chronic autoimmune disease of unknown etiology, primarily affecting the synovial joints however characterized by a broad spectrum of extra-articular manifestations [4]. The clinical course of RA is variable, ranging from mild to severe disease, which can potentially lead to joint damage, chronic disability and early mortality [5].

The development of autoimmunity linked to the

disease can serve as evidence that the immune system initiated RA. Anti-citrullinated protein antigens (ACPAs) are produced long before the occurrence of rheumatoid arthritis symptoms [6]. Induced citrullination of proteins can consequently cause a breach of peripheral immune tolerance to self-antigens, leading to inflammation and autoimmunity [7]. Opportunistic infection with *T. gondii* is an increasing problem in association with inflammatory rheumatoid [8].

There is growing interest in exploring the link between infection with this parasite and autoimmune diseases, given the propensity of *T. gondii* infection to occur in immunocompromised patients [9]. Human Leukocyte Antigen-G gene was first described by Geraghty et al. [10] as a member of the non-classical Class I. Class I molecule is a heterodimer (beta-2 microglobulin) consisting of a heavy chain and a light chain. The HLA-G gene is located within the major histocompatibility complex on the chromosome 6 and can bind receptors on several immune cell types and inhibit the function or induce apoptosis of CD8 natural killer and T cells [11]. HLA-G-positive antigen-presenting cells are also potent inhibitors of CD4 T cell proliferation. Therefore, a role of HLA-G could be expected in autoimmune diseases and in particular in rheumatoid arthritis [12].

HLA-G exerts its function through interaction with KIR2DL4 (killer-cell immunoglobulin-like receptor) and this signaling is supported by the fact that NK cells represent more than 80% of decidual lymphocytes [13]. Functionally, KIR2DL4 has been reported to be an inhibitory receptor for peripheral NK cells and uterine NK cells [14]. Additionally, KIR2DL4 has been shown to activate cytokine production, but not cytotoxicity, in resting NK cells from peripheral blood [15].

In this study we aimed to investigate the effect of *Toxoplasma gondii* on some critical immunological markers (sHLA-G and KIR2DL4) proteins in rheumatoid arthritis Iraqi patients.

Materials and Methods

A total of 150 blood samples were collected from women with rheumatoid arthritis (RA) attends the Rheumatology Department in Baghdad Teaching Hospital in Baghdad governorate. Out of those 150 RA patients only 50 patients were including in this study. This selection was done according to exclusion of any patient had negative RF or CRP or ACCP test for homogeneity. Blood samples were also collected from 60 apparently healthy women. Disease activity of rheumatoid arthritis is measured by DAS (disease activity score). All patients and healthy control women were subjected to IgM and IgG Toxo ELISA kit (Foresight-UAS) to investigate any toxoplasmosis infection. Women without toxoplasmosis were considered a negative control group while women with toxoplasmosis were considered a positive control group. All the samples succumbed to diagnostic tests with HLA-G and KIR2DL4 ELISA kits (Bioassay Technology Laboratory-China).

Statistical analysis

The Statistical Analysis System – SAS 2018 program was used to detect the effect of difference factors in study parameters. Least Significant Difference – LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01) probability in this study.

Results

All the patient samples were subjected to Toxo ELISA tests (IgM and IgG), the result elucidated that only 25 RA patients were infected with chronic toxoplasmosis (RA +ve Toxo +ve IgG), as well as 25 apparently healthy donor were also with chronic toxoplasmosis while the other 35 were healthy (control negative) (Tab. 1).

In relation to other results, the current results showed that treated RA patients without coinfection with *Toxoplasma* had the highest significant (10.72±1.35 pg/ml) increase in the level of sHLA-G in comparison to RA untreated patients (6.37±0.54 pg/ml).

However, treated RA co-infected patients with *Toxoplasma* showed high significant increase of sHLA-G (7.72±0.64 pg/ml) in comparison to co-infected untreated (4.09±0.61 pg/ml) (Tab. 2).

Additionally when looking to the result of sHLA-G level in patients with toxoplasmosis only. The result obtained that its level was significantly ($P \leq 0.01$) increased (6.68±0.78 pg/ml) in comparison to control group (3.79±0.31 pg/ml).

On other hand, the current results showed that treated RA patients without *Toxoplasma* had the highest significant (17.29±2.2 pg/ml) increase level of KIR2DL4 protein in comparison to RA without

Table 1. The percentage distribution of RA patients and healthy donors according to Toxo ELISA test (IgM and IgG)

Test subject	Total	Toxo IgM acute		Toxo IgG chronic		Negative samples	
		No	(%)	No	(%)	No	(%)
RA patient	50	0	0	25	50	25	50
Healthy control	60	0	0	25	41.6	35	58.33
Chi-square	–	–	NS	–	2.603 NS	–	2.557 NS

Table 2. The levels of sHLA-G in studied samples

Group		No	Mean ± SE HLA-G (pg/ml)
RA patients (n=25)	Treated	15	10.72±1.35a
	Untreated	10	6.37±0.54 bc
Toxoplasmosis+RA (n=25)	Treated	15	7.72±0.64 b
	Untreated	10	4.09±0.61cd
Toxoplasmosis (n=25)		25	6.68±0.78 bc
Control (n=35)		35	3.79±0.31 d
LSD value		–	2.072 **
P-value		–	0.0038

Explanations: different letters in columns indecte a significantly difference; ** $P \leq 0.01$

Toxoplasma untreated patients (14.462±2.4 pg/ml). While, treated RA patients with *Toxoplasma* showed high significant increase ($P \leq 0.01$) of KIR2DL4 (13.077±2.21 pg/ml) in comparison to RA untreated patients (9.85±2.45 pg/ml) (Tab. 3).

Also the results of the level of KIR2DL4 in patients with toxoplasmosis only showed significantly decrease ($P \leq 0.01$) (8.47±2.28 pg/ml) in comparison to control group (10.88±1.54 pg/ml) (Tab. 3).

The severity of disease infection was clearly appeared in co-infected patients (RA+Toxo) through the high levels of Das 28 in both groups as seen in table 4, which was (4.35±0.05). While patients woman with toxoplasmosis only and with RA patients without *Toxoplasma* had the lower

Table 3. The levels of KIR2DL4 receptor in studied samples

Group		No	Mean ± SE KIR2DL4 (pg/ml)
RA patients (n=25)	Treated	15	17.29±2.21 a
	Untreated	10	14.462±2.45 ab
Toxoplasmosis+RA (n=25)	Treated	15	13.07±4.34 ab
	Untreated	10	9.85±1.92 c
Toxoplasmosis (n=25)		25	8.47±2.28 c
Control (n=35)		35	10.88±1.54 bc
LSD value		–	4.037 **
P-value		–	0.0001

Explanations: means having with the different letters in same column differed significantly; ** $P \leq 0.01$

Table 4. The levels of DAS 28 in studied samples

Group		No	Mean ± SE DAS 28
RA patients (n=25)	Treated	15	3.76±0.02 a
	Untreated	10	4.10±0.14 a
Toxoplasmosis+RA (n=25)	Treated	15	3.70±0.10 a
	Untreated	10	4.90±0.14 a
Toxoplasmosis (n=25)		25	1.79±0.05 b
Control (n=35)		35	1.52±0.05 b
LSD value		–	1.082 **
P-value		–	0.0097

Explanations: see table 3

infection according to DAS 28 score (1.97±0.05) and (3.93±2.3), respectively. Statistical analysis proved the existence of significant differences ($P \leq 0.01$) between studied groups.

Discussion

T. gondii infection is more likely in patients with rheumatic diseases due to changes in innate and adaptive immune responses. Patients with RA were found to be extremely vulnerable to *T. gondii* infection, especially during periods of immunosuppression following treatment. As a result, any decrease in the body's defenses against infection puts arthritis patients at risk. Actually, rheumatoid arthritis patients present with abnormal regulatory network in the immune response, which includes HLA-G gene [16]. The current study found that serum sHLA-G protein concentrations were significantly lower in untreated RA patients, which agreed with previous findings by Rizzo et al. [17] and Shakir and Al-Qadhi [18]. Reduced sHLA-G concentrations may cause persistent inflammatory cell activation and contribute to disease progression [18].

In rheumatoid arthritis, chronic synovial inflammation causes progressive damage to articular cartilage and bone, eventually resulting in disability. For RA patients, a number of disease-modifying antirheumatic drugs (DMARDs) are available. Conventional synthetic DMARDs, particularly biological DMARDs, have been demonstrated to effectively inhibit joint destruction in RA.

DMARDs include many drugs, such as methotrexate, hydroxychloroquine, sulfasalazine, and leflunomide. The DMARD therapy has ability to modify HLA-G secretion by enhances HLA-G secretion by peripheral blood mononuclear cells [19]. Thus it was evident why the level of HLA-G in the treated groups (RA and RA co-infection) was higher than in the untreated groups. Furthermore, DMARDs cause an increase in IL-10 levels. Because IL-10 is a potent antagonist of IFN-γ, it could be a key cytokine involved in mediating *T. gondii*-induced immunosuppression in the infected host. As a result, drugs used to treat RA cause an increase in HLA-G levels and increasing the risk of toxoplasmosis.

Despite the fact that biologic drugs have been shown to be effective in the treatment of RA patients, long-term monitoring of patients treated with TNF-inhibitors revealed serious side effects, particularly intracellular microorganism infection (tuberculosis, histoplasmosis, toxoplasmosis). Many RA patients have been documented in recent years that developed toxoplasmic chorioretinitis and cerebral toxoplasmosis while being treated with anti-TNF medications [4].

In addition, the recruitment of activated monocytes and/or other myeloid cells, which can secrete large amounts of sHLA-G, may be linked to the elevated release of sHLA-G in the inflamed synovium of RA patients. As a result, the migration of a large number of sHLA-G secreting cells from the peripheral blood to the inflamed synovium may have a role in the lower levels of sHLA-G found in sera [20].

As mentioned, HLA-G antigens are characterized by anti-inflammatory and immuno-inhibitory functions, thus the presence of these molecules could affect disease activity [19].

Rizzo et al. [17] were cleared the relation between sHLA-G and RA when they mentioned that the decreased sHLA-G serum concentrations may lead to a chronic activation of inflammatory cells and contribute to the development of autoimmune diseases and here in the present study the result showed that when there was a lower amount of sHLA-G there was a severe infection with one of autoimmune disease (RA).

Toxoplasma infection causes NK cells to activate, and sHLA-G has been shown to maintain immunologic tolerance by interacting with dNK inhibitory receptors to inhibit the cytotoxicity of NK cells, which results in lower sHLA-G levels in RA-coinfected patients compared to RA patients without *Toxoplasma* infection.

More recently, in another cohort, in Iraq by Shakir and AL-Qadhi [18] also agreed with the current study result when they mentioned that the sHLA-G level in toxoplasmosis group and toxo-coinfection with RA was higher than its level in control group .

HLA-G is characterized by tolerogenic functions, inducing apoptosis of activated CD8+ T cells, acting on T regulatory cells, modulating the activity of natural killer cells and of dendritic cells and blocking allo-cytotoxic T lymphocyte response. These immuno-regulatory functions are mediated by the interaction of HLA-G molecules with specific inhibitory receptors: ILT2 (LILRB1/ CD85j), ILT4 (LILRB2/CD85d), CD8 and KIR2DL4 (CD158d) expressed by immune cells [21].

sHLA-G can regulate adaptive and innate immunity by interaction with T or B lymphocytes and NK cells or polymorphonuclear cells. HLA-G can inhibit all proliferation and immunoglobulin production, it can also alter antigen present to T lymphocytes by inhibiting dendritic cell function and maturation [22,23].

Increased expression of KIR2DL4, an inhibitory receptor on NK cells, induced by elevated sHLA-G as an immunosuppressive molecule, suppressing NK cell cytotoxicity in response to *T. gondii* infection. Excessive immunological tolerance could result from increased sHLA-G and NK suppression, which could be associated to *T. gondii* immunity escape [14]. This explains why RA patients have high levels of both HLA-G and KIR2DL4 together and it was established that following *T. gondii* infection, the level of KIR2DL4 was reduced in the current study. But this result was not deals with another study by Liu et al. [24] who referred to increase the levels of KIR2DL4 receptor with *Toxoplasma* infection.

The current findings suggested that *Toxoplasma* may play a role in increasing inflammation and NK cytotoxicity in RA patients, as evidenced by lower levels of both sHLA-G and KIR2DL4 in RA patients infected with *Toxoplasma* compared to RA patients without toxoplasmosis, and that the parasite may play a role in blocking the KIR2DL4 receptor.

In the current study the *Toxoplasma* seropositive RA patients presented with severe clinical manifestations such as morning stiffness, higher numbers of tender and swollen joints and higher DAS28 scores than *Toxoplasma* seronegative patients. The disturbances in the immunological response interfere with parasite control and thereby have an impact on the clinical course. RA patients typically have circulating auto-antibodies, rheumatoid factor and anti-cyclic citrullinated peptide. These antibodies were significantly linked to *Toxoplasma* positivity, particularly when presenting in high titres. The obtained results were supported by several clinical and experimental studies which found that microbial infections can induce and/or exaggerate the symptoms of arthritis [25].

While the rest of groups (toxoplasmosis and control) showed lower DAS rates than RA *Toxoplasma* seronegative and RA *Toxoplasma* seropositive, this may happened due to the absence of citrullinate protein, and that trigger the immune response and inflammation.

This research provided the role of HLA-G's activity of upregulating the amount of inhibitory receptor KIR2DL4 to limit NK cell activation in both of toxoplasmosis and rheumatoid arthritis patients.

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