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The effects of toxoplasmosis infection on the levels of IL-18 and anti-ds-DNA antibodies in SLE patients

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***Abstract*---**The present study was set to demonstrate the prevalence of toxoplasmosis infection and its effects on patients with systemic lupus erythematosus (SLE) through determining their serum levels of anti-dsDNA and IL-18 antibodies. For this purpose, the sera from 132 SLE and/or toxoplasmosis patients and 30 healthy women, were collected. The study sample was divided into four groups of SLE, toxoplasmosis, SLE coinfected with toxoplasmosis, and healthy control. Anti-Toxoplasma IgG antibodies were examined for all the samples using ELISA kit. The results showed a high mean level of anti-Toxoplasma IgG among SLE patients coinfected with toxoplasmosis (104.8792±12.31585pg/ml) in comparison to that in toxoplasmosis patients (91.1705±12.57746pg/ml). However, the mean value of anti-dsDNA antibodies showed significant (P≤0.01) elevation in SLE patients and SLE patients coinfected with toxoplasmosis (71.2134±9.22131pg/ml and 72.3699±9.67917 pg/ml, respectively), compared to those infected with toxoplasmosis only and healthy control. Furthermore, the IL-18 mean value in the sera of the studied patients showed significant (P≤0.01) elevation (418.4000±43.18072pg/ml) in SLE patients with toxoplasmosis in comparison to SLE patients (354.4400±35.29257pg/ml). The present investigation suggests that the levels of anti-Toxoplasma IgG antibodies seem to increase in patients with SLE. In addition, IL-18 levels were elevated in patients with toxoplasmosis, but those of anti-dsDNA were not affected.

***Keywords*---**toxoplasma gondii, systemic lupus erythematosus, Interleukin-18, anti-ds DNA antibodies.

Introduction

Toxoplasmosis is an opportunistic zoonotic infection that affects all warm-blooded animals, including humans [1]. *T. gondii* infection is currently believed to be asymptomatic in immunocompetent patients [2]. Immunocompromised people are more susceptible to toxoplasmosis infection as a result of a compromised immune system [3]. *T. gondii* infection demonstrated immunomodulatory impact on host cells. Numerous studies have been conducted to determine if *T. gondii* may have a therapeutic effect on a variety of autoimmune diseases. *T. gondii* has been involved in the induction of autoimmune disorders like lupus erythematosus, according to a previous systematic review and meta-analysis [4]. Immunity against *T. gondii* is mediated by a complex immune response including inflammatory cells, lymphocytes, macrophages, and cytokines [5].

Systemic lupus erythematosus (SLE) is a complex autoimmune disease marked by pathogenic autoantibodies and excessive inflammation. It is most prevalent in young women throughout their childbearing years, although it may occur in children at any age, elderly, and males. SLE's inflammatory responses may affect several organs. Its severity varies considerably, ranging from asymptomatic to severe and irreparable tissue damage and death [6]. T lymphocytes receive autoantigens from antigen-presenting cells (APCs), such as macrophages and dendritic cells, resulting in their induction and production of proinflammatory cytokines. Autoantibodies are produced by B cells, which aggregate into immunological complexes that deposit on and damage organs [7, 8]. Opportunistic organisms like *T. gondii*, which are typically seen in immunocompromised patients, occur at high levels in SLE patients. Significant evidence suggests that these infections, both symptomatic and asymptomatic, may cause chronic immune system activation and autoimmunity [9].

Interleukin-18 (IL-18) is a cytokine with pro-inflammatory properties and a member in the superfamily of interleukin-1 (IL-1), which has some physiological properties and activities with other family members, such as IL-1α and IL-1β5. IL-18 exerts its effects on dendritic cells, monocytes, macrophages, neutrophils, and epithelial cells, impairing their survival, maturation, and cytokine production [10]. SLE pathogenesis is effected by cytokines in a variety of ways, and their balance dictates disease activity. Cytokines seem to be involved in both disease control and organs damaged in SLE patients [11]. High disease activity is related with the presence of IL-18 in SLE patients' peripheral blood mononuclear cells (PBMCs) [12].

Numerous autoimmune disorders result in the formation of autoantibodies. About 70 % of SLE patients have anti-dsDNA antibodies, compared to less than 0.5 % of healthy individuals and patients with other autoimmune diseases. Their presence in the blood of lupus patients for many years prior to the beginning of the disease suggests that they may have a role in disease development [13]. As a result, anti-dsDNA antibodies may detect a range of self-antigens, triggering immune system response, cell death, and tissue fibrosis, ultimately destroying organs and target cells [14].

Materials and Methods

One hundred and thirty-two blood samples were collected from women who attended Baghdad Teaching Hospital from November 2021 to April 2022, ninety of

them were diagnosed with SLE. The patients age ranged from 19 to 53 years. In addition, 30 blood samples were collected from healthy women, as a control group, with an age range from 19 to 58 years. Five milliliter (ml) of venous blood was taken from each subject in sterile conditions. The collected serum was stored at -20 °C until used.

All samples were first tested for anti-*Toxoplasma* IgG using ELISA (Abnova, Germany). Accordiing to the results of this test, the study sample was divided into four groups:
G_1 (50 SLE patients with seronegative toxoplasmosis)
G_2 (40 SLE patients with seropositive toxoplasmosis)
G_3 (42 Seropositive toxoplasmosis patients)
G_4 (30 Healthy control)
Afterwards, the serum levels of IL-18 (Human Interleukin-18, Boster antibody and ELISA experts, USA) and anti-dsDNA antibodies (Abnova ELISA, Germany) were tested for all groups.
The study design was accepted by the Research Ethical Committee of the College of Science, University of Baghdad (No. CSEC/1021/0056).
The obtained results and data were tabulated in excel. The Statistical Analysis System- SAS (2012) program was used to investigate the parameters of this study. To determine significant differences between the means of compared groups, t-Test was used

Result
The levels of anti-*Toxoplasma* IgG antibodies in the studied groups

Anti-*Toxoplasma* IgG antibodies were measured by ELISA technique for all the 132 subjects, 90 of which were diagnosed with SLE disease. Anti- *Toxoplasma* IgG antibodies mean level in G_2 patients (SLE co-infected with toxoplasmosis) was 104.8792±12.31585 pg/ml, while the level in G_3 patients (with toxoplasmosis only) was 91.1705±12.57746 pg/ml. The mean level in SLE patients (G_1) was 8.1415±1.10575 pg/ml, whereas in the control group (G_4) it was 5.9115±0.83191pg/ml, as shown in table 1. According to the results, there were highly significant ($P \leq 0.01$) differences in the mean serum level of anti- *Toxoplasma* IgG antibodies between subjects in G_2 and G_3 compared to G_1 and G_4 groups.

Table 1
The mean levels of anti-*Toxoplasma* IgG antibodies in the sera of the studied groups

Groups	Means ±SD Anti-*Toxoplasma* IgG antibodies(pg/ml)
G_1	8.1415±1.10575^{c}
G_2	104.8792±12.31585^{a}
G_3	91.1705±12.57746^{b}
G_4	5.9115±0.83191^{c}

Means with different letters in the same column differed significantly **(P≤0.01).

Anti-ds-DNA antibodies mean levels in the sera of studied groups

The current results showed that the mean level of anti-dsDNA antibodies in G2 sera was 72.3699±9.67917 pg/ml, which represents the highest value as compared to the other groups. Meanwhile, patients in group G1 showed a mean value of 71.2134±9.22131 pg/ml), whereas those in groups G3 and G4 recorded 15.2495±1.53938 12.0344±1.31750 pg/ml, respectively. The results indicated significant (P≤0.01) differences between G1and G2 compared with G3 and G4 groups, as shown in table 2.

Both groups G1 and G2 groups revealed high levels of serum anti-dsDNA antibodies because they both included SLE patients, which are usually characterized by the presence of high levels of these antibodies according to the activity of the disease. Also, the results showed that the infection of SLE patients with toxoplasmosis did not affect the level of these antibodies.

Table 2
Anti-ds-DNA antibodies mean levels in the sera of studied subjects

Groups	Means ±SD Anti-ds-DNA antibodies (pg/ml)
G_1	71.2134±9.22131[a]
G_2	72.3699±9.67917[a]
G_3	15.2495±1.53938[b]
G_4	12.0344±1.31750[b]

Means with different letters in the same column differed significantly **(P≤0.01).

Interleukin-18 mean level in the sera of the studied groups

The results showed that the mean values of IL-18 was 418.4000±43.18072 pg/ml in the sera of G_2 subjects and 354.4400±35.29257 pg/ml in G_1. However, G_3 had a mean value of 239.6000±25.74168 pg/ml, while the healthy control group showed a value of 80.8667±9.26965 pg/ml. The mean level of IL-18 in the sera of G_2 was significantly (P≤0.01) higher than that in G_1. SLE patient's levels of IL-18 were also significantly (P≤0.01) higher than those in G_3 and G_4. On the other hand, toxoplasmosis patients (G_3) had significantly (P≤0.01) higher level of IL-18 compared with healthy control, as revealed in table 3.

Table 3
Interleukin-18 mean level in the sera of the studied subjects.

Groups	Means±SD IL-18(pg/ml)
G_1	354.4400±35.29257[b]
G_2	418.4000±43.18072[a]
G_3	239.6000±25.74168[c]
G_4	80.8667±9.26965[d]

Means with different letters in the same column differed significantly **(P≤0.01).

Discussion

The major diagnostic approach for *Toxoplasma* infection is the identification of *Toxoplasma*-specific antibodies. For IgG antibodies, the enzyme-specific immunoassays are the most frequently utilized diagnostics nowadays [15].*T. gondii* is assumed to infect 25 to 30 % of the world's human population. In Iraq, compared to the 1980s when the incidental rate of toxoplasmosis among women was 2%, the incidence of toxoplasmosis has increased by more than 40% over the past recent years [16].The high prevalence of *T. gondii* infections in women has been related to their increased exposure to Toxoplasma through interaction with cats and environmental contact [17].However, infection rates of *T. gondii* are dependent on health and socioeconomic standards [18].The current results agreed with a previous research which revealed that all *T. gondii*-exposed patients had significant levels of IgG antibodies directed against antigens in the chronic phase of infection [19].

Our results also agree with those of a previous research which showed that patients with autoimmune disease, such as SLE, are more likely to be infected with toxoplasmosis. This explains the high prevalence of *T. gondii* among SLE patients [20].Other reports demonstrated that *T. gondii* seropositivity rate in SLE patients was 60%. Likewise, rheumatoid arthritis and SLE patients tested positive for toxoplasmosis-specific antibodies [21, 22]. dsDNA antibodies were initially discovered and quantified 55 years ago and are currently widely used to support the diagnosis and treatment of SLE because they correlate with disease activity and may be involved in its development [23]. Anti-dsDNA antibodies are produced due to a combination of many reasons, including abnormalities of dendritic cells, B cells, or T cells, along with the absence of DNase, which makes cells unable to clean the released nuclear materials [24].

Anti-dsDNA antibodies are considered as a critical marker for the diagnosis and classification of SLE. Moreover, the fluctuating titers of these antibodies reflect the activity of disease of many patients; a rising titer could be predictive of disease relapse[25]. According to previous studies, higher levels of anti-dsDNA antibodies were detected in patients with active SLE [26].Another previous study, in line

with ours, showed that anti-dsDNA antibodies levels appear to remain similar in sera of SLE patients after being infected with T. gondii [27].

It was observed in a previous study that IL-18 levels were elevated in all SLE patients [28].A significant difference in interleukin-18 levels in SLE patients compared to normal people was also observed [29]. These results support those of the current study. Also, it appears that the infection of SLE patients with toxoplasmosis led to an increase in the level of IL-18 compared with non- infected SLE group. IL-18 is a pro-inflammatory cytokine that stimulates both innate and acquired immune responses. IL-18 is expressed in immune cells, such as natural killer (NK) cells, dendritic cells, and macrophages, and its expression is increased in SLE and associated with disease activity. Furthermore, IL-18 has been shown to accelerate the development of lupus-like autoimmune disease in MRL/LPR-deficient mice., Further, since IL-18 is located on the chromosome near the SLE susceptibility locus, it is believed to contribute to genetic susceptibility to SLE [30].The elevated titers of IL-18 were seen in SLE patients primarily due to the development of lupus nephritis (LN). Also, serum IL-18 levels were shown to be significantly correlated with anti-ds-DNA titers in SLE patients who were in the active stage of the disease [31].

IL-18 was reported to contribute to IFN-γ production induced by immunopathological features in the small intestine after oral infection with *T. gondii.* IL-18 decreased the number of parasites isolated from the tissue and corresponded with an increase in NK cell activity [32].The data presented by Cai and colleagues agreed with ours, demonstrating that IL-18 serum levels were increased with toxoplasmosis infection. They also showed that IL-12 mediated the resistance to *T. gondii* induced by IL-18, which is a potent enhancer, although it could enhance resistance to toxoplasmosis independently of IL-12. Another previous study showed that the oral infection with toxoplasmosis allowed to simultaneously monitor proinflammatory (development of Th1-type immuno-pathology) and antimicrobial (parasite replication) effects of IL-18, using knockout mice deficient in IL-18 [33].IL-18 was demonstrated as the key mediator of small intestinal immunopathology, whereas mice deficient in IL-18 partially control parasite replication and up to 50% of mice survive the infection [34].

In Conclusions, *Toxoplasma gondii* had high incidence among women confirmed by measuring anti-*Toxoplasma* IgG antibodies even for lupus erythematous cases affirming SLE patients more susceptible to toxoplasmosis infection. Combination of infection with SLE disease ,anti-ds-DNA levels doesn't seem to be effected by the parasite. Moreover, IL-18 level switched from normal to high levels in present cases , mostly in SLE cases usually associated with disease activity and development of lupus nephritis ,thus a highest levels in SLE cases with toxoplasmosis due to its involvement in Toxoplasmosis resistance.

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