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INFLUENCE OF TOXOPLASMA GONDII INFECTION ON INTERLEUKIN-23 LEVELS IN IRAQI DIABETIC TYPE 2 PATIENTS

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Abstract

Toxoplasma gondii is an obligate protozoan intracellular parasite with a global distribution which can infect approximately all warm blooded animals and humans. Diabetes mellitus is a abnormal metabolism with impropriety hyperglycemia due to either a minimizing in the biologic effectiveness of insulin or an absolute lacking of insulin secretion, or both. Type 2 diabetes mellitus is ordinarily caused via insulin resistance, and frequently together with progressive defect in insulin secretion. IL-23 is a member of the IL-12 family, which stimulate the responder function of Th17 cells to enhance inflammatory responses. The major objective of this study is to comprehend the effect of toxoplasmosis on IL-23 levels in Iraqi diabetic type 2 patients and understand its role in chronic infection. The study conducted on 109 blood specimen of Iraqi diabetic type 2 patients compared with 80 blood specimens of non-diabetic control with age mean 49.9 ± 1.29, the entire specimens gathered from private lab in Baghdad, Iraq, through the period March to June 2022. The results where the group of diabetic patients has the highest level of glucose in their blood in diabetic diagnostic tests 7.9 ± 0.178 HbA1C, 174.55 ± 3.96 mg/dl FBG and 216.89 ± 4.96 mg/dl RBG respectively. Furthermore, 51/109 (26.98%) cases of the group of diabetic patients were seropositive to anti-Toxoplasma IgG antibody, its titer was 34.95 ± 7.5 UI/mL in CMIA followed by 30/80 (15.87%) samples of non-diabetic control have 32.7 ± 8.45 UI/mL for the same antibody in the same assay recording significant differences when compared to the other groups. Whereas, all samples were seronegative in CMIA for anti-Toxoplasma IgM antibody. The group of diabetic patients with toxoplasmosis has the highest level of IL-23 in ELISA 1265.76 ± 79.37 pg/ml followed by the group of diabetic patients 684.69 ± 34.59 pg/ml then the group of positive toxoplasmosis control 547.51 ± 8.13 pg/ml. Whereas, lowest level of interleukin was appeared in healthy control 518.39 ± 7.48 pg/ml with highly significant differences. This study concludes that of T. gondii infection has an effect on the IL-23 in diabetic type 2 patients.

Keywords: T. gondii, DMT2, IgM, IgG and IL-23

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan considered one of the most common parasites that infect warm-blooded animals. Humans are involved as intermediate hosts for the parasite, while cats serve as the final hosts. Humans are primarily infected postnatally by ingesting infectious oocysts from the environment or by eating raw or undercooked meat, suggesting tissue cysts or other modes of infection. However, *T. gondii* usually causes mild, self-limiting infections in immunocompetent individuals. However, *T. gondii* can vertically infect the fetus during pregnancy and cause a range of clinical manifestations in its offspring, leading to miscarriage, fetal abnormalities and prenatal death in pregnant women and for some immunocompromised patients with organ transplant recipients or AIDS. Diagnosis of this parasitic infection in humans can be made by various immunological and molecular methods. (1, 2, 3, 4).

Diabetes can be identified as a metabolic disorder characterized by persistent hyperglycemia resulting from abnormalities in insulin secretion, insulin action, or both. The effects of diabetes include long-term damage, dysfunction, and failure of various

organs (5, 6). There are two types of diabetes. Insulin dependent (type I) and insulin independent (type II). The second type of diabetes accounts for 95% of diabetes cases worldwide and its etiology may be primarily related to obesity and inadequate use of insulin in the body (7, 8).

However, due to its innate acute inflammatory response and antigen-specific adaptive immunity, *T. gondii* can infect and replicate in any nucleated host cell inducing the formation of various inflammatory markers that enhance chronic inflammation. (9). A minor interpretation of the association between *T. gondii* and T2DM might be that diabetics are more susceptible to parasitic infections due to reduced arterial blood flow, possible weakened immune system and neuropathy (10).

Inflammatory response is one of the major pathologies of *Toxoplasma*-infected hosts. Infection with *T. gondii* stimulates increased production of various pro-inflammatory cytokines such as TNF-α, IFN-γ and IL-1β, which can lead to the development of insulitis (11, 12). In addition, these pro-inflammatory cytokines have direct cytotoxic effects and may induce destruction of pancreatic beta cells. In some studies, *T. gondii* can disrupt the balance of pro- inflammatory and anti-inflammatory cytokines in diabetic patients and cause damage to host tissues. It may directly attack pancreatic tissue, destroy beta cells, and increase the risk of acute and chronic pancreatitis leading to diabetes (13, 14, 15).

IL-23 is a pro-inflammatory cytokine that promotes the pro-inflammatory function of a subset of memory T cells called Th17 characterized by the production of the cytokine IL-17 (16, 17). IL-23 cooperates with IL-6 to differentiate Th17 cells. In the face of chronic inflammation, stimulated dendritic cells and macrophages produce IL-23, which promotes the development of Th17 cells (18). This study was conducted to demonstrate the effector role of *T. gondii* on IL-23 levels in her type 2 diabetic patient in Iraq and to assess the association between them.

MATERIALS AND METHODS

1. Subjects and Samples

The study enrolled 189 cases from March to June 2022, with 80 samples collected from non-diabetic outpatient clinics as controls and type 2 diagnosed by experts attending a private laboratory in Baghdad. 109 samples collected from diabetic patients were included. In Iraq, the age range for all cases was from 15 to 85 years (49.9±1.29). Five ml of venous blood was drawn from each sample then put it in a gel tube and at 3000 rounds per minute (rpm) for 10 minutes to aspirate the serum. Serum was separated and utilized for several diabetes tests, measuring of anti-*Toxoplasma* IgM/IgG antibodies, and evaluating of IL-23 levels.

2. Diabetes mellitus diagnosis

Blood glucose assessed by fasting test followed by random test through using Glucose architect kit (Abbott GmbH, Germany) with assessing glycated hemoglobin level via using hemoglobin A1C Architect kit (Abbott GmbH, Germany) depending on the manufacturer's instructions.

3. *T. gondii* diagnosis

Chemiluminescent microparticles immunoassay (CMIA) Architect Toxo IgM/G kit (Abbott GmbH, Germany) used to detect mean titer of anti-*Toxoplasma* IgM/IgG antibodies according to the manufacturer's procedures.

4. Evaluation of IL-23 levels

The mean titers of IL-23 were evaluated via using Human IL-23 (Interleukin 23) Sandwich Enzyme-linked Immunosorbent assay (ELISA) kit (mybiosource Inc., USA) according to the manufacturer's instructions.

Statistical Analysis

Studying the effect of difference factors in current study parameters were performed by using the Statistical Analysis System- SAS (19) program. Least significant difference –LSD test (Analysis of Variation-ANOVA) was utilized to critical compare between mean titers. Significant comparing between percentage (0.05 and 0.01) probability was accomplished by using Chi-square test.

RESULTS AND DISCUSSION

Opportunistic infections such as toxoplasmosis are more common because people with diabetes are more susceptible to infection than healthy people. Diabetes increases host susceptibility and increases the risk of transmission to various infectious diseases (20). The results of this study showed that the diabetic group had the highest glycated hemoglobin, fasting and random test blood glucose levels compared to the non-diabetic group, as shown in Table (1).

Table 1: Mean values of HbA1C, FBG and RBG tests in the studied groups.

Groups	Total No. of samples for each group	Mean ± SE of HbA1C (Glycated Hemoglobin)	Upper Value	Lower Value	Mean ± SE of FBG mg/dl (Fasting blood glucose)	Upper Value	Lower Value	Mean ± SE of RBG mg/dl (Random blood glucose)	Upper Value	Lower Value
Diabetic Patients	109	7.9 ± 0.178	15.5	5.3	174.55 ± 3.96	300	120	216.89 ± 4.96	410	125
Non-Diabetic Control	80	4.98 ± 0.044	5.4	4.3	96.65 ± 0.749	98	81	160.25 ± 2.69	195	109
LSD value		1.667 *			26.381 **			31.093 **		
P-value		0.0252			0.0063			0.0058		
Significant * (P≤0.05), Highly significant ** (P≤0.01).										
Reference range of HbA1C Normal < 5.7 Prediabetes 5.7 – 6.4 Diabetes ≥ 6.5.		**Reference range of FBG** Normal < 100 mg/dl. Prediabetes 101 – 125 mg/dl. Diabetes ≥ 126.								
Reference range of RBG: over 200 mg/dl after two hours refers to diabetes.										

The results of this study resemble to the results of Waheed *et al.,* (21) that demonstrated the group of Iraqi diabetic type 2 patients 30/50 has the highest levels of glucose in FBG test 130.8±16.575 mg/dl and HbA1C test 7.993±0.646 in comparison with Iraqi non-diabetic group 20/50 that has 83.8±9.689 and 4.68±0.484 in FBG and HbA1C tests. HbA1C is preferred due to it is more time versatile and informative in long-term circumstances. The HbAlc test is recently become one of the best ways to check diabetes to be under control.

Their benchmarks have been stabilized in last years. It has been concluded that FBG is more precise than HbA1C (22, 23, 24).

However, the study groups divided into four groups referring to the mean titer of anti-*Toxoplasma* IgG as the following: diabetic patients infected with toxoplasmosis, diabetic patients only, non-diabetic individuals infected with toxoplasmosis considered as a positive control and healthy individuals considered as a negative control. In addition, all specimens of diabetic patients and non-diabetic control have negative response for anti-*Toxoplasma* IgM with significant differences.

Table (2) revealed that the group of diabetic patients 51/109 has the highest mean titer of anti-*Toxoplasma* IgG antibody 34.95 ± 7.5 UI/mL followed by non-diabetic positive control 32.7 ± 8.45 UI/mL (30/80) with significant differences when measured by chemiluminescent microparticle immunoassay (CMIA).

Tenter *et al.,* (25) explained that the high levels of antibody IgG are due to the reason that the antibody IgG is one of the most important components of the humoral immune response in controlling the parasite and reducing its distribution. However, This IgG antibodies detected usually within 1-2 weeks after infection and reach the highest concentrations in the period (6-8) weeks and then progressively decline over a year or two and low levels may continuous a lifetime.

Also, it is found that the levels of antibody IgM decrease faster than the levels of antibody IgG in immunized people after long injury may be for many years. In addition, the IgG antibody has the capability to substitute large molecules in the absence of antibody generators therefore the half-life of the IgG antibody is greater than half the life of the IgM antibody (26).

Table 2: Seroprevalence of Anti-*Toxoplasma* IgM and IgG antibodies in study samples

Groups	Total No. of samples for each group	Mean ± SE of Toxo IgG UI/mL	Upper Value	Lower Value	Mean ± SE of Toxo IgM UI/mL	Upper Value	Lower Value
Diabetic patients with toxoplasmosis	51 (26.98%)	34.95 ± 7.5 a	217	0.6	0.082 ± 0.0052 a	0.2	0.02
Diabetic patients	58 (30.69%)	0.024 ±0.058 b	2.3	0.0	0.072 ± 0.003 a	0.16	0.02
Toxoplasmosis patients (control positive)	30 (15.87%)	32.7 ± 8.45 a	230	5.8	0.10 ± 0.04 b	0.19	0.01
Healthy individuals (control negative)	50 (26.46%)	0.38 ± 0.055 b	2.5	0.0	0.042 ± 0.005 ab	0.13	0.01
LSD value		0.218 *			0.0595 *		
P-value		0.0392			0.0478		
Means having with the various letters in same column differes remarkably. Significant * (P≤0.05), Highly significant ** (P≤0.01).							
Reference range of Toxo IgM: Primary (acute) infection ≥ 0.6. **Reference range of Toxo IgG**: Secondary (chronic) infection ≥ 3.0							

The current results similar to the results of Al-Khafajii (27) that demonstrated 22/45 (48.88%) of diabetic patients were seropositive for anti-*Toxoplasma* IgG antibody however 28/55 (50.9%) of non-diabetic control were seropositive for the same antibody.

Besides, the recent results matched with the results of of Al-Aubaidi *et al.* (28) that shown 47/100 of diabetic patients have seropositive for *Toxoplasma* IgG antibody in

comparison with healthy control that shown seronegative for the same antibody in the same test with highly significant differences.

Referring to the above results, this declares that the risk factor of toxoplasmosis in diabetic patients was higher than in healthy controls. Hence, patients infected with toxoplasmosis may be increases at the risk to develop diabetic rather than uninfected individuals (29).

Thirty samples of each group used to evaluate the levels of IL-23 which demonstrated in table (3) that the group of diabetic patients with toxoplasmosis has the highest level of IL-23, whereas the group of healthy control has the lowest level of interleukin with highly significant differences (P≤0.01).

Table 3: The mean levels of IL-23 (pg/ml) in the sera of the studied groups

Groups	No. of samples for each group	Mean ± SE pg/mL	Upper Value	Lower Value
Diabetic patients with toxoplasmosis	30	1265.76 ± 79.37 a	1932.3	558.6
Diabetic patients	30	684.69 ± 34.59 b	922.87	443.05
Toxoplasmosis patients (control positive)	30	547.51 ± 8.13 c	621.94	500.21
Healthy individuals (control negative)	30	518.39 ± 7.48 c	593.2	455.05
LSD value	102.58 **			
P-value	0.0001			
Means having with the various letters in same column differed significantly. Highly significant ** (P≤0.01).				

Nevertheless, highly significant differences appeared when the group of diabetic patients with toxoplasmosis is compared multiply with the other studied groups. Interleukin-23 (IL-23) is a cytokine whose identified deeply impacted theories around chronic inflammation and autoimmunity, it's associated to the IL-12 family of cytokines, belong to the same portion of the IL-6 superfamily.

The IL-12 family consist of four heterodimeric cytokines that involvement in the sequence homology (30, 31). The first identification member was IL-12, consists of two subunits, p35 and p40. IL-23 contributes homology with granulocyte colony stimulating factor (G-CSF) and IL-6.

This interleukin is expressed basically through the macrophages and dendritic cells. The IL-23R is surfaced on memory T cells, macrophages, NKT cells, naive T cells and DCs when stimulated through TGF-β and IL-6 (32, 33).

The above results of IL-23 nearly similar to the results of Assim and Saheb (34) that illustrated the group of Iraqi women with breast cancer and toxoplasmosis has the highest level of IL-23. While, the lowest level of interleukin was in the healthy control group in all age range.

Interleukin (IL)-23 plays a role in moderate degrees of type 2 diabetes relevant inflammation (35). In chronic infections, antigens induce macrophages, dendritic cells and IL-23 production that stimulates the production of IL-17.

Besides, IL-23 raises the production of IL-1, IL-6 and tumor necrosing factor (TNF)-α in the autocrine/paracrine pathway (36). IL-23 has a critical role in immunity against infection with *T. gondii* (37), there were some findings around production of the IL-12

and IL-23 in macrophages and DCs after *T. gondii* infection, these findings referred that IL-12, not IL-23, plays a main role in resistance to toxoplasmosis however in the lacking of IL-12, IL-23 can supply a restricted mechanisms of resistance to the infection (38, 39, 40).

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