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Seroprevalence of Toxoplasmosis Antibodies among Diabetes Mellitus Patients and Assessment some Biochemical Markers

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Abstract

Toxoplasma gondii is a protozoan intracellular coccidian protozoan parasite. Latent toxoplasmosis threat to immunocompetent individuals. Diabetic patients are more susceptible to infect with toxoplasmosis due to their low level of immunity response. The purpose of this research is to define the association between toxoplasmosis and diabetes mellitus and detection serum levels of chemokines (monocyte chemoattractant protien-1 and transforming growth factor-β) in diabetic patients infected with toxoplasmosis. Serum samples were collected from 120 diabetic patients and 50 healthy individuals as a control group from the Imamein Kadhimein Medical City in Baghdad. In order to diagnose diabetes, fasting and random blood glucose tests were used. The diagnosis of toxoplasmosis was done by using toxo IgM and IgG immulite torch assay. Chemokines levels were measured by ELISA method. The results clarified that all samples were seronegative for IgM antibodies while 50 (41.67%) diabetic patients were seropositive for IgG antibodies and 70 (58.33%) diabetic patients and 50 healthy controls were seronegative for IgG antibodies. Serum level of MCP-1 was recorded an increase in a group of diabetic patients infected with toxoplasmosis 169.66 ± 131.35 pg/ml with highly significant differences ($P < 0.001$) also TGF-β level was increased in a group of diabetic patients infected with toxoplasmosis 0.53 ± 0.242 pg/ml with highly significant differences ($P \leq 0.0001$) when compare with the studied groups.

Keywords: Toxoplasmosis, Diabetes mellitus (type 2), MCP-1, TGF-β.

1. Introduction

Toxoplasmosis is an important zoonotic disease caused by intracellular obligate protozoan called *Toxoplasma gondii*. This parasite has two stages in the life cycle, asexual stage occurs in any warm-blooded animal (intermediate host) and sexual stage occurs in felines (definitive host) [1]. *T.gondii* has three morphological stages: tachyzoites that multiplied rapidly in any intermediate host cell and in non-intestinal epithelial cells of the felines, bradyzoites that multiplied slowly within a tissue cyst and oocysts develops in the intestine of felines. Humans are infected by contaminated water, half-cooked meat and vegetables that are not disinfected [2].

Its distribution in worldwide and about 20-90% of world adult population has serum *Toxoplasma* antibodies. Several factors affected on the distribution of this parasite such as cultural levels, age, residency, sanitation, nutritional habits, gender, modes and cat bearing houses [3]. Parasites will proliferate within the host cells lysing them, which they can disseminate through the body by blood circulation and infect any cells. Fetus through the placenta of infected mothers can acquire infection; congenital toxoplasmosis may cause serious damage to the fetus [4].

Diabetes like Human Immunodeficiency Virus (HIV) or other immunodeficiency disorders can lead to opportunistic infections. There are several reports demonstrating that diabetic patients have an increased susceptibility to many specific infections such as toxoplasmosis [5]. Diabetes mellitus is a chronic disorder characterized by persistent hyperglycemia with disturbances in fat, carbohydrate, and protein metabolism causing from abnormalities in secretion or action of insulin [6]. It classified according to American Diabetes Association in to four clinical classes: Insulin dependent diabetes mellitus (T1D), Non-insulin dependent diabetes mellitus (T2D), Gestational diabetes mellitus (GDM) and other specific types of diabetes [7]. About 10% of diabetes distribution was type 1 and gestational diabetes while 90% was diabetes type 2. T2D characterized by abnormally high blood glucose resulting from relative insulin deficiency. General symptoms of diabetes include frequent urination, increased, unexplained weight loss and thirst, also may include feeling tired and increased hunger [8].

Diabetic patients are more susceptible to infect with toxoplasmosis due to low level immunity response [9]. In immunocompetent individuals 90% of toxoplasmosis infection is asymptomatic [10]. Infection with *T. gondii* causes humoral immunity (H.I.) and cell mediated immunity (C.M.I.) components of adaptive responses are needed because this parasite in intracellular pathogen, but also move through extracellular space to find new host cell [11]. The object of this study is to assess the correlation between toxoplasmosis and diabetes and assess determine the serum levels of chemokines MCP-1 and TGF- β in diabetic patients infected with toxoplasmosis and control also clarify their correlation with other clinical parameters.

2. Materials and Methods

One hundred and twenty blood samples of diabetic patients were collected, their age ranging from 12 to76 years with mean 50.9 ± 13.8 from the Imamein Kadhimein Medical City in Baghdad, Iraq, during the period from September until the end of December 2016, after that they were diagnosed by a specialized physician and 50 blood samples of healthy individuals collected as controls. Five milliliters of venous blood were collected using 5 ml disposable syringe and then transferred immediately to gel tube and left to clot for 15 minutes at room temperature (20-25°C), samples were centrifuged at 2500 to 3000 rpm for 10 min period to separate serum. Some of serum used to diagnose diabetes by using fasting and random blood glucose tests (LABKIT, Spain) then *T. gondii* infection was diagnosed by immulite torch assay (Flex reagent cartridge IgM and IgG, Siemens, Germany) and the residual of serum stored in eppendrof tubes until used at -20C^{o} for measuring chemokines levels. One hundred samples were selected to detection chemokine levels by Sandwich ELISA method: According to *T.gondii* diagnostic test the samples were divided to 50 patients have diabetes with toxoplasmosis, 50 patients have diabetes without toxoplasmosis, and 25 healthy controls.

Monocyte chemoattractant protien-1 and transforming growth factor-β levels have been estimated by (ELISA) technique enzyme immunoassay using the manufacturer instruction as supplied with the kit from peprotech, USA.

The data are presented as a Mean ± SD. Chi-square test was used to analyze the results. Least significant difference (LSD) test was used to significant compare. A p-value of < 0.05 was statistically significant considered.

3. Results

The present results of 170 samples of Toxo IgM in immulite 2000 torch assay in table (1) clarified that all of 170 (100%) samples are seronegative response for previous assay. No significant differences showed in Toxo IgM immulite 2000 torch assay.

Table (1): Distribution of *T. gondii* infection according to Toxo IgM IU/ml immulite torch assays in studied groups

Diagnosis	Response for toxoplasmosis	Diabetic Patients		Control		P-Value
		No.	%	No.	%	Sig.(*)
Flex reagent cartridge IgM	+ ve	0	0.00	0	0.00	1.00 NS
	- ve	120	100	50	100	
Total		120		50		

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Table (2) illustrates that 50 patients from 120 (41.67%) patients are seropositive for Toxo IgG in immulite 2000 torch assay which infected diabetes and toxoplasmosis and 70 (58.33%)

patients are seronegative for Toxo IgG in immulite 2000 torch assay which infected diabetes only, while 50 (100%) volunteers have negative response for this assay. Highly significant differences explained in this assay ($P \leq 0.0001$).

Table (2): Distribution of *T. gondii* infection according to Toxo IgG IU/ml immulite torch assays in studied groups

Diagnosis	Response for toxoplasmosis	Diabetic Patients		Control		P-Value Sig.(*)
		No.	%	No.	%	
Flex reagent cartridge IgG	+ ve	50	41.67	0	0.00	0.0001 **
	- ve	70	58.33	50	100	
Total		120		50		

Table (3) show comparisons of IgM levels in studied groups estimated as IU/ml such group of patients that suffering from diabetes and infected with toxoplasmosis has highest level 0.386 ± 0.03 then group of diabetic patients has 0.366 ± 0.02 and control group has less level of IgM 0.437 ± 0.03 with highly significant differences, also the same table show the highest and lowest value of IgM level for each group, while table (4) show comparisons of IgG levels in studied groups estimated by IU/ml. Group of diabetic patients with toxoplasmosis has highest level of IgG 106.17 ± 12.67 followed by diabetic patients group has 3.68 ± 0.09 and healthy volunteers group has less level 3.89 ± 0.13 with highly significant differences ($P \leq 0.0001$), also the same table illustrates highest and less value of IgG level for each group.

Table (3): Levels of Toxo IgM (IU/ml) in studied groups with statistical description.

Groups	No.	Mean IU/ml	Std. Dev.	Std. Error	Lower Value	Upper Value
Diabetic patients infected with toxoplasmosis	50	0.386	0.21	0.03	0.1	0.8
Diabetic patients	70	0.366	0.16	0.02	0.1	0.8
Control	50	0.437	0.17	0.03	0.2	0.8
LSD-Value	15.371**					
P-Value	0.0001					

Table (4): Levels of Toxo IgG (IU/ml) in studied groups with statistical description

Groups	No.	Mean IU/ml	Std. Dev.	Std. Error	Lower Value	Upper Value
Diabetic patients infected with toxoplasmosis	50	106.17	89.65	12.67	13.4	260
Diabetic patients	70	3.68	0.82	0.09	2.3	5.2
Control	50	3.89	0.73	0.13	3.1	5.1
LSD-Value	21.873**					
P-Value	0.0001					

Table (5) showed that group of diabetes patients has highest value of fasting blood glucose 188.31 ± 72.12 pg/ml, group of diabetes patients with toxoplasmosis has 155.42 ± 5.84 pg/ml and healthy control group has lowest level 111.41 ± 10.48 pg/ml with highly significant differences among studied groups ($P \leq 0.0001$).

Table (5): Levels of FBG in studied groups with their comparisons.

Groups	No.	Mean Pg/ml	Std. Dev.	Std. Error	LSD-Value	P-Value
Diabetes patients with toxoplasmosis	50	155.42	51.84	7.33	24.617	0.0001**
Diabetes Patients	70	188.31	72.12	8.55		
Control	50	111.41	10.48	1.94		

While results of table (6) illustrate the highest value of random blood glucose estimated in Pg/ml of diabetes patients 246.31 ± 87.39 pg/ml then followed by 205.05 ± 70.72 pg/ml of diabetes patients with toxoplasmosis and 141.58 ± 14.30 of healthy control individuals, highly significant differences appear in these results ($P \leq 0.0001$).

Table (6): Levels of RBG in studied groups with their comparisons.

Groups	No.	Mean Pg/ml	Std. Dev.	Std. Error	LSD-Value	P-Value
Diabetes patients with toxoplasmosis	50	205.05	70.72	10.00	30.884	0.0001**
Diabetes Patients	70	246.31	87.39	10.37		
Control	50	141.58	14.30	2.65		

Table (7) clarified the results of MCP-1 levels in studied groups that's found in mean ± SD of diabetic patients with toxoplasmosis 169.66 ± 131.35 pg/ml ranging from 29.39 to 449.16 pg/ml and it was higher than diabetic patients group 119.77 ± 95.78 pg/ml ranging from 20.44 to 296.61 pg/ml in comparison with healthy control group 62.31 ± 54.25 pg/ml ranging from 8.7 to 173.82 pg/ml, also highly significant differences was recorded ($P < 0.01$).

Table (7): Concentrations of MCP-1 (pg/ml) in sera of studied groups

Groups	No.	Mean (Pg/ml)	Std. Dev.	Std. Error	Lower Value	Upper Value
Diabetes patients with toxoplasmosis	50	169.66	131.35	18.57	29.39	449.16
Diabetes patients	50	119.77	95.78	21.41	20.44	296.61
Control	25	62.31	54.25	12.13	8.79	173.82
LSD-Value	62.511**					
P-Value	0.0017					

Results of table (8) revealed that the mean difference between diabetic patients with and without toxoplasmosis was significant 49.89 pg/ml, as well as mean difference between diabetic patients with toxoplasmosis and healthy control was highly significant 107.35 pg/ml, also mean difference between diabetic patients without toxoplasmosis and healthy control was highly significant 57.46 pg/ml.

Table (8): Comparisons of MCP-1 levels (pg/ml) estimated in sera of studied groups.

Parameter	Group 1	Group 2	Mean Diff.	P-Value	Sig. (*)
MCP-1 Concentration (Pg/ml)	Diabetes patients with toxoplasmosis	Diabetes patients	49.89	0.0382	*
		Control	107.35	0.0001	**
	Diabetes patients	Control	57.46	0.0026	**

Table (9), illustrates the results of TGF-β levels in studied groups that found the mean ± SD of diabetic patients with toxoplasmosis 0.242 ± 0.53 pg/ml ranging from 0.149 to 0.995pg/ml has higher TGF-β level than diabetic patients group 0.23 ± 0.076 pg/ml their ranging values from 0.133 to 0.346 pg/ml in comparison with healthy control group 0.102 ± 0.12 their values from 0.012 to 0.352 pg/ml, with highly significant differences shown ($P \leq 0.0001$).

Table (9): Concentrations of TGF-β (pg/ml) in sera of studied group.

Groups	No.	Mean (Pg/ml)	Std. Dev.	Std. Error	Lower Value	Upper Value
Diabetes patients with toxoplasmosis	50	0.53	0.242	0.034	0.149	0.995
Diabetes patients	50	0.23	0.076	0.017	0.133	0.346
Control	25	0.12	0.102	0.022	0.012	0.352
LSD-Value	0.1074**					
P-Value	0.0001					

Table (10) illustrates the mean difference (pg/ml) among studied groups have a significant difference ($P < 0.01$).

Table (10): Comparisons of TGF-β levels (pg/ml) estimated in sera of studied groups.

Parameter	Group 1	Group 2	Mean Diff.	P-Value	Sig. (*)
TGF-β Concentration (Pg/ml)	Diabetes patients with toxoplasmosis	Diabetes patients	0.3	0.0037	**
		Control	0.41	0.0001	**
	Diabetes patients	Control	0.11	0.0492	*

4. Discussion

A possible correlation between toxoplasmosis and diabetes involve clinical significances, shedding light on the complicated pathogenesis of diabetes. Generally, the current hypothesis supposes that toxoplasmosis increases the capability to infect with diabetes and, on the other hand, diabetic patients are abler to infect with toxoplasmosis [12,13].

Newly, the immulite 2000 torch assay, an automated *Toxoplasma* Quantitative IgM and IgG test, has been presented, which measures Toxo IgM and IgG in International Units per milliliter (IU/ml) of serum. This assay is simple, comparatively inexpensive and rapid needful 60–90 minutes for completion [14].

The previous results of IgM and IgG Abs agreement with Shirbazou *et al.* [5] study that showed the prevalence of IgG and IgM Abs in diabetic patients were (56.6%) and (2.4%) while in control were (22.4%) and (1.6%) respectively, as well as agreement with results of Gokce *et al.* [15] that studied serologic detection of anti-*Toxoplasma* infection in 91 diabetic patients and 93 healthy control which found the prevalence of IgG Ab of *T. gondii* was 55 (60.43%) while in healthy control was 36 (38.7%), also compatible with results of El-Awady *et al.* [14] that studied seroprevalence of toxoplasmosis in 110 diabetic pregnant women and 110 non diabetic pregnant women which found 47 (42.7%) of diabetic pregnant women were seropositive for anti-*Toxoplasma* IgG and 3 (2.7%) seropositive for IgM Ab as well as 24 (21.81%) of healthy non diabetic pregnant women were seropositive for IgG Ab but there is no detection for IgM Ab.

These findings discovered the prevalence rate of IgG Ab was directly related with duration of diabetes because of the weakened immune system of diabetic patients which also proposed that toxoplasmosis patients are more susceptible to be diabetics than those without. Demolition of the pancreas occurs in three stages of *T. gondii*:

1. Hyperactive stage (hyper-period) in which β-cell obliteration of nerve cells and less interference in the insect in a hyperactive state of the pancreas, sometimes insulin secretion is excessive, frequently resulting in low or a too low blood sugar, this stage is often occurring during adolescence.
2. Disordered stage (compensatory stage), in which neurons and pancreatic β-cells have a great amount of damage, under normal conditions, insulin secretion will be insufficient, the body will begin the compensative function. So, when few in the disordered state, this stage of insulin secretion over time.
3. Decline stage (recession), in which nerve cells and β-cells destruction of more compensatory function reach to its limits.

There are a number of factors such as duration of diabetes, severity of noninfectious complications, abortion, optical disease, comorbidity, co-infection and meat consumption which can lead to acute infection in diabetic patients and also severe complications of disease (16).

The present results similar to results of Modrek *et al.* [16] that investigate of IgG and IgM in 205 serum samples of diabetics in Ali Asghar Hospital in Zahedan (southeastern Iran) with age (13 – 60) years which found 131 diabetic patients had fasting blood glucose levels between 121 - 300 mg/dL that 79 diabetics have anti *Toxoplasma* IgG (63.2%) and 52 diabetics have anti- *Toxoplasma* IgM (71.3%) with significant differences ($P < 0.05$).

Monocyte chemoattractant protein (MCP)-1 is a chemokine with chemoattractant characteristics for monocytes, memory T cells, mast cells, natural killer cells, and basophils [17]. It is involved in the cellular recruitment and activation of several leukocytes such as monocytes/macrophages, polymorphonuclear cells, and lymphocyte infiltration to the sites of infection [18]. MCP-1 secretion may participate to the monocytes and lymphocytes recruitment and therefore contribute in the control of T. gondii pathogenesis and its infection [19]. Study of Ali [20] revealed that low levels of MCP-1 (CCL2) were observed in pregnant women with acute toxoplasmosis because live tachyzoite forms of *T. gondii* inhibited the

synthesis of MCP-1[21]. Study of Tuaillon *et al.* [17] referred to the well-known metabolic variations that happen in diabetic and normal subjects. Healthy individuals produce an "accelerated starvation" in the fasting situation, with a prior and more profound hypoglycemia and an increased level of fasting insulin; however diabetic patients elucidate elevated concentrations of fasting insulin [22]. Furthermore, MCP-1 has angiogenic effect on endothelial cells, thus it can participate to the development and remodeling of adipose tissues [23]. High glucose processing on endothelial cells isolated from diabetic subjects produced in about 40-70% increase releasing of MCP-1 [24]. Elevated serum MCP-1 levels could contribute to the onset and progression of several complications in diabetes. Thus, serum MCP-1 may serve as a biomarker of inflammatory activity and helps in early detection and intervention of diabetic complications [25].

Results of MCP-1 levels weree similar to results of Waheed *et al.* [25] that studied MCP-1 level in 30 type 2 diabetic patints and 20 healthy control which found mean ± SD of patients 48.87 ± 12.345 pg/ml higher than in healthy control 38.8 ± 8.994 pg/ml with significant differences, while dissimilar with results of Ali [20] that studied some cytokines in 65 pregnant women infected with acute toxoplasmosis and 50 healthy married women (non-pregnant) which found that mean ± SE of pregnant women 14.46 ± 1.38 pg/ml higher than healthy non pregnant women 11.25 ± 0.24 pg/ml without any significant differences.

The results of this study showed that TGF-β levels rise in diabetic patients with toxoplasmosis more than in other groups that's resemble to the results of Waheed *et al.* [25] that studied TGF-β in 30 type 2 diabetic patients and 20 healthy control which found mean ± SD of patients 19.67 ± 6.586 pg/ml higher than in healthy control 13.6 ± 4.641 pg/ml without significant difference, as well as resemble to the results of Ali [20] that studied some cytokines in 65 pregnant women infected with acute toxoplasmosis and 50 healthy married women (non-pregnant) which found the level in pregnant women was 20.99 ± 4.13 pg/ml while in healthy control 1.52 ± 0.05 with significant differences.

TGF-β is a cytokine which plays key roles in the regulation of immune cell functions [20]. This cytokine leads to inhibition of B and T lymphocytes proliferation and induces homeostasis [26]. TGF-β also contributes to tissue remodeling which occurs after infections and injuries, this cytokine contributes to development of Th17 and T-regulatory lymphocytes, activation and [27]. TGF-β secreted from monocytes in T2DM patients have been reported [28]. Also Telejko assumed that TGF-β can be a key agent accountable for the modifications in circulating MCP-1 levels, but the action and secretion of this chemokine in diabetes mellitus require more researches [22]. TGF-β stimulates the synthesis of acute phase proteins and has been found to be over expressed in the glomeruli of patients with diabetes nephropathy, also involved in the induction of extracellular matrix production [29]. Lyons *et al*. [30] found constituent expression of TGF-β in the eye, but furthermore they discovered that ocular toxoplasmosis considerably increases transcripts TGF-β levels, whereas this increase in TGF-β levels can help to preserve immune privilege and to decrease damage caused from inflammation, also it can have useful effects on the survival of *T. gondii* and its proliferation. For instance, TGF-β has been revealed not only to decrease the capability of NK cells, a main source of IFN-γ, to react to *T. gondii* but also to markedly downregulate

functions of macrophage, including their capability to create nitric oxide and indolamine [31]. The production of TGF-β is stimulated after infection with *T. gondii* [32]. TGF-β raises *Toxoplasma* replication on cultured retinal cells, proposing that cytokine might be implicated in immunopathological phenomena [33].

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