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Some Immunological aspects in Diabetic Type 2 Patients Infected with Toxoplasmosis

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ABSTRACT

Toxoplasmosis is a disease caused by an obligate intracellular parasite called *Toxoplasma gondii*. Many reports revealed that diabetic patients are more susceptible to infect with toxoplasmosis. The aim of this study is to clarify the relation between toxoplasmosis and diabetes mellitus type 2 with measuring the levels of some cytokines like interferon-γ and interleukin-6 (IFN-γ and IL-6) in diabetic patients infected with toxoplasmosis as compared with healthy controls. One hundred and twenty serum samples of diabetic type 2 patients and 50 healthy individuals were collected from the Imamein Kadhimein Medical City in Baghdad. Diabetes diagnosis by fasting and random glucose tests, also the diagnosis of toxoplasmosis infection was done by using Toxo IgM nd IgG antibodies immulite torch assay. Cytokines levels were measured by Sandwich ELISA method. The results explained that all samples have negative results for Toxo IgM antibodies while 50 (41.67%) diabetes samples have positive results for Toxo IgG antibodies and 70 (58.33%) diabetes samples have negative for Toxo IgG antibodies as compared with 50 healthy controls that have negative results for IgG antibodies. IFN-γ levels was recorded increasing in a group of diabetes patients infected with toxoplasmosis 558.66 ± 271.01 pg/ml with a highly significant differences as well as IL-6 levels was recorded increasing in a group of diabetic patients infected with toxoplasmosis 145.428 ± 31.13 pg/ml with a highly significant differences ($P < 0.0001$) when compared with other study groups.

Keywords: Toxoplasmosis, Diabetes mellitus (type 2), IFN- γ, IL-6.

1. INTRODUCTION

Toxoplasma gondii is an obligate parasite belongs to the phylum Apicomplexa. It caused an important disease called toxoplasmosis. This parasite has two stages in life cycle, asexual stage takes place in any warm blooded animals (intermediate host) like birds and other mammals and sexual stage takes place in felines (definitive host) (1). *T.gondii* has three morphological phases: tachyzoites that multiplied in any cell of intermediate host ,bradyzoites multiplied within tissue cysts and oocysts evolved in the intestine of definitive host (2). It transmitted by contaminated water or food with oocysts or through placenta, polluted needles and blood transfusion (3). This parasite invades the adjoining cells of the host and replicates causing rupture and death with focal necrosis surrounded by an acute inflammatory response. The distribution of *T.gondii* depends on many factors like age, gender, sanitation, modes and cat bearing houses (4).

Diabetes mellitus (DM) is a metabolic disease caused by elevated blood glucose resulting from a defect in secretion and action of insulin. There are four clinical types of diabetes according to American Diabetes Association: type 1 (insulin dependent diabetes mellitus), type 2 (insulin independent diabetes mellitus), gestational diabetes mellitus (GDM) and other specific types (5). T2D represents worldwide distributed that occurs after the age of 35-40 years but recently it is also occurring in children (6). It is chronic inflammatory disorder due to chronic hyperglycemia that responsibly for multiple micro and macro vascular complications like nephropathies, retinopathies and cardiovascular diseases (7). The symptoms of T2D are

commonly like increased hunger, weight loss, thirst, feeling tired and frequent urination (8).

Several disorders can lead to infect with toxoplasmosis such as Human Immunodeficiency Virus (HIV), diabetes, and other immunodeficiency disorders, many reports elucidated that patients with diabetes have more susceptibility to infect with toxoplasmosis (9).

About 90% of immunocompetent patients have asymptomatic toxoplasmosis infection. Humoral immunity (H.I.) and cell mediated immunity (C.M.I) will be activated against *T.gondii* that infect intracellular and may pass through extracellular space to find new host cell (10).

The purpose of this study is to clarify the association between toxoplasmosis and diabetes mellitus and measure the levels of interferon-γ and interleukin-6 (IFN-γ and IL-6) in diabetic patients compared with healthy individuals, also illustrate their relation with other clinical parameters like IgM and IgG antibodies, fasting and random blood glucose.

2. MATERIALS AND METHODS

2.1 Selection of Patients

This study included collect 120 samples of diabetic patients after diagnosed by endocrinologist at the Imamein Kadhimein Medical City during September until the end of December 2016 with age ranging 12-76 years with mean 50.9 ± 13.8. Five milliliters of venous blood were gathered by a disposable syringe then immediately transferred to gel tube and left to clot at room temperature (20-25°C) for 15 minutes. These samples centrifuged at 2500-3000 rpm for 10 minutes to separate serum then some of serum used for diabetes diagnoses (Glucose MR, Linear, Spain) then immulite torch assay used for *T.gondii* diagnosis (Flex reagent cartridge IgM and IgG, Siemens, Germany) and the residual of serum stored in eppendrof tubes until used at -20Cº for measuring cytokines levels study. One hundred samples used for detection cytokines levels by Sandwich ELISA method: a group of 50 samples of diabetic patients infected with toxoplasmosis and a group of 50 samples of diabetic patients only as well as a group of 25 samples of healthy individuals selected as control.

2.2 Sandwich ELISA method

Sandwich ELISA technique enzyme immunoassay used for measuring the levels of interferon-γ and interleukin-6 by using the manufacturer directives as provide with the kit from peprotech, USA.

2.3 Statistical analysis

Chi-square test was used to analyze the results, also least significant difference (LSD) test used for significant compare. Statistical significant that used for this study was a P-value < 0.05

3. RESULTS AND 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 more able to infect with toxoplasmosis (9).

A group of diabetic patients only has the highest level of fasting and random blood glucose as compared with other groups with highly significant differences as shown in table (1) and (2).

Table 1: 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		

Table 2: 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		

The present results were similar to Modrek *et al.* (11) results that investigated 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).

Consequently, several experimental evidence have been evaluated and suggested as plausible

pathophysiological mechanisms to illuminate this correlation, including:

1. Infected white blood cells assimilate improved migratory feature, causing the easier distribution of *Toxoplasma* in body organs, such pancreas.
2. A clinically visible autoimmune procedure could be ignited by *Toxoplasma* infection, trending immune machinery across auto antibody production, for example against Langerhans islets cells.
3. A probability, is that *T.gondii* itself may attack and destroy pancreatic cells directly, initiating pancreatitis and more importantly, diabetes (12).
4. Creation of reactive oxygen species (ROS) and nitric oxide (NO) is stimulated by diabetes, and these agents, as stimulant for intracellular pathogens, can reactivate latent, cysts of parasites, over starting acute infection (13).
5. Given the incapability of neutrophils to correctly achieve phagocytosis and intracellular killing in progressive stage of diabetes, there may be raise in responsiveness to intracellular pathogens like *Candida* and *Toxoplasma*.
6. The opsonization activity and leukocyte cytotoxicity of diabetic patients need for removal of pathogens are extensively subsided; therefore they would be more prone for opportunistic infections (14).

Table (3) revealed that all samples of diabetes have negative results for anti-*Toxoplasma* IgM without any significant differences. Table (4) illustrates that a group of healthy control has the highest value of IgM Abs levels as compared with other groups with highly significant differences.

Table 3: 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 Sig.(*)
		No.	%	No.	%	
Flex reagent cartridge IgM	+ ve	0	0.00	0	0.00	1.00 NS
	- ve	120	100	50	100	
Total		120		50		

Table 4: 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				

While table (5) clarified that 50 samples of diabetes have seropositive for anti-*Toxoplasma* IgG and 70 samples have seronegative for IgG Abs as well as a group of diabetic patients infected with toxoplasmosis has highest levels of IgG Abs as compared with other groups with highly significant differences (table 6).

Table 5: 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 6: 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				

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 (15).

The previous results of IgM and IgG Abs agreed with the study of El-Awady *et al.* (15) 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. as well as agreement with results of Shirbazou *et al.* (16) 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, also compatible with results of Gokce *et al.* (17) 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%).

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 occurs 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.

Decline stage (recession), in which nerve cells and β-cells destruction of more compensatory function reach to its limits (11).

The results of table (7) clarified that a group of diabetic patients infected with toxoplasmosis has the highest levels of IFN-γ with highly significant differences when compared with other groups.

Table 7: Concentrations of IFN-γ (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	558.66	271.01	38.32	108.66	989.20
Diabetes patients	50	429.91	218.79	48.92	137.85	878.56
Control	25	215.01	124.81	27.90	52.26	462.75
LSD-Value	132.11**					
P-Value	0.0001					

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

Parameter	Group 1	Group 2	Mean Diff.	P-Value	Sig. (*)
IFN-γ Concentration (Pg/ml)	Diabetes patients with toxoplasmosis	Diabetes patients	428.75	0.0461	*
		Control	643.65	0.0001	**
	Diabetes patients	Control	214.9	0.0371	*

Interferon-γ (IFN-γ) is a glycosylated protein of 25 kDa. Firstly, it was assumed that CD4+Th1 lymphocytes, CD8+ cytotoxic lymphocytes, and NK cells entirely produce IFN-γ. The biological action of IFN-γ is a dimmer, and its main role is the stimulation of macrophages to increase phagocytosis, tumoricidal properties, and intracellular killing of pathogens, specifically bacteria and fungi. Moreover, it stimulates macrophage production of many inflammatory mediators and reactive oxygen and nitrogen intermediates (20, 21).

IFN-γ was found to be indicating in diabetes pathogenesis and the frequency of the low IFN-γ creation allele (A-allele) was found considerably higher in T2D compared to controls. Published data appeared an increased IFN-γ level in diabetic type 2 patients without nephropathies; therefore it looked that the improvement of IFN-γ level is maybe associated to the diabetes more than nephropathies (18, 22). This suggests that *T. gondii* is an opportunistic intracellular parasite that induces a highly strong type-1 cytokine response such as IFN-γ and IL-2 (23) during first infection as a result of early T cell in addition to NK cell stimulation (24). Another study stated that IFN-γ levels creased in acute stage of infection, while decreased in chronic stage (25).

A group of patients with diabetes and toxoplasmosis has the highest level of IL-6 with significant differences when compared with a group of control as shown in table (9), (10).

Table 9: Concentrations of IL-6 (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	145.428	31.13	4.40	99.2	271.8
Diabetes patients	50	128.81	28.25	6.31	85.6	183
Control	25	108.88	11.19	2.50	76.8	127
LSD-Value	15.371					
P-Value	0.0001**					

Table 10: Comparisons of IL-6 levels (pg/ml) estimated in sera of studied groups.

Parameter	Group 1	Group 2	Mean Diff.	P-Value	Sig. (*)
IL-6 Concentration (Pg/ml)	Diabetes patients with toxoplasmosis	Diabetes patients	16.618	0.092	NS
		Control	36.548	0.0278	*
	Diabetes patients	Control	-19.93	0.096	NS

In this study, the association of IL-6 and toxoplasmosis in diabetic patients as shown in the results, there is strong association of IL-6 and toxoplasmosis in diabetic patients P ≤ 0.0001, as well as a study by Vidhate *et al.,* (26) that clarified the association of IL-6 with diabetes in Indian population from Navi Mumbai by studying 18 samples of diabetic patients type 2 and 18 person apparently healthy control which found the mean of IL-6 in serum of diabetic patients higher than mean of healthy control, also another study by Matowicka-Karna *et al.,* (27) clarified strong relationship between IL-6 and toxoplasmosis that studied IL-6 in serum of 52 sample of women infected with *T. gondii* and 40 sample of healthy women which found the mean level of IL-6 in *T. gondii* infected patients was being twice as high as the mean value of healthy control, with significant differences (P ≤ 0.0001).

According to previous results, the description of the influence of IL-6 in diabetes type 2 was the macrophages accumulation in adipose tissue, the general origin of macrophages and adipocytes, the predominant existence of peripheral mononuclear cells and apoptotic beta cells by themselves look to be the cause of inflammation in diabetic type 2 patients (28). The main function of IL-6 is the participation in the immune response across the action on B-lymphocytes; it is an intermediary responsible for increased cytotoxic activity of NK cells and the creation of acute phase proteins. IL-6 is an early, sensitive, though nonspecific and indicator of inflammatory situations (29). The management of an anti-IL-6 monoclonal antibody in a model of murine toxoplasmic encephalitis decreases the inflammatory lesions and amount of cysts in the brains of these mice. In murine toxoplasmosis, a gradual increase in serum IL-6 is related with clinical signs (30). Furthermore, IL-6 has been recognized as a factor responsible for the growth of insulin resistance, also it has been correlated with the occurrence of type 2 diabetes and other complications of diabetes such as diabetic retinopathy (31)

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