Detection Antibodies IgM, IgG and Determination Levels of IL-33 in Iraqi Diabetic Type 2 Patients Infected with *Toxoplasma Gondii*

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Abstract

Toxoplasma gondii is an obligate intracellular protozoan that causes a disease called toxoplasmosis which has the capability to pass its both life cycle parts asexual and sexual in the final host (cats)and also through intermediate vertebrate hosts including humans. Diabetes mellitus is a metabolic disorder that there is high blood sugar level over a prolonged period. This research included one hundred and twenty blood samples that collected from diabetic patients after diagnosing them by endocrinologist at the Imamein Kadhimein Medical City during August until the end of December 2016 with age ranging 12-76 years with mean 50.9 ± 13.8. Toxoplasmosis diagnoses was occurred by using Toxo IgG and IgM antibodies immulite torch assay while diabetes diagnosis by fasting blood sugar tests also measuring level of IL-33 in samples was done by sandwich ELISA method that showed a group of diabetic patients infected with toxoplasmosis have the highest level of IL-33. The results showed that a group of diabetic patients have the highest level of glucose in diabetes test (188.31 pg/ml)while a group of diabetic patients infected with toxoplasmosis have the highest level of IgG (106.17 IU/ml)while all samples have seronegative results for Toxo IgMalso the highest level of IL-33 showed ina group of diabetic patients infected with toxoplasmosis (858.84 pg/ml) while a group of diabetic patients not infected with toxoplasmosis have (556.67 pg/ml) and control group have (315.58pg/ml).

Keywords: *Toxoplasma gondii, IgM, IgG, Diabetes mellitus type 2, IL-33.*

Introduction

Toxoplasma gondii is an obligate intracellular protozoan that causes a disease called toxoplasmosis[(1)]. It has the ability to pass both asexual and sexual parts of it slife cycle in feline (cat) as the final host, and a wide spectrum of warm-blooded vertebrate hosts including humansas intermediate hosts [(2)]. It is a zoonotic disease according to *T. gondii* is one of the most common parasites of animals[(3)]. *T. gondii* passes through three shapes: tachyzoites responsible for active infection, bradyzoites found in tissue cysts and sporozoites found in environmentally resistant oocysts formed after the sexual part of the life cycle [(4)].

Diabetes Mellitus (DM), commonly referred to as diabetes, is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period [(5)]. Type 2 is representing about 90% of all cases of the disease; it is characterized by the coexistence of insulin deficiency and the peripheral effects of the hormone [(6)].

Diabetic patients are more amenability to infect with *T. gondii* due to their low level immune response, toxoplasmosis and diabetes may pave the way to each

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other; the occurrence of necrotic lesions in the pancreas of the experimental animals infected with *T. gondii* may indicate that toxoplasmosis paves the way to diabetes as the involvement of the pancreas can lead to the inhibition of insulin secretion and consequently the establishment of diabetes [7].

The object of this research as the following:

1. Determine levels of glucose by using FBS test.
2. Detection antibodies IgM and IgG of *T. gondii* infection.
3. Determine levels of interleukin-33 in Iraqi diabetic type 2 patients infected and controluninfected individuals in order to understand the relationship between these diseases.

Materials and Method

Selection of Patients: This research included one hundred and twenty blood samples collected from diabetic patients after diagnosed by endocrinologist at the Imamein Kadhimein Medical City during August until the end of December 2016 with age ranging 12-76 years with mean 50.9 ± 13.8. Six milliliter of venous blood was gathered by using 10 ml disposable syringe. These samples were immediately carried to gel tube and left to clot at room temperature (20-25°C) for 15 – 20 minutes, after cloting it was centrifuged at 2500 to 3000 rpm for 10 min period to separate serum that used for fast blood sugar diagnoses (Glucose MR, Linear, Spain) then immulite torch assay used for *T.gondii* diagnosis (Flex reagent cartridge IgG, Siemens, Germany) while the remaining serum distributed in tightly closed eppendrof tubes by micropipette, each eppendrof included (0.25 ml) and then the tubes were stored at -20ºC until used. For detection cytokines levels by Sandwich ELISA method, one hundred diabetic samples used and divided as: 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.

Sandwich ELISA method: Sandwich ELISA technique enzyme immunoassay used for measuring the levels of interleukin-33 by using the manufacturer directives as provide with the kit from peprotech, USA.

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.

Results and Discussion

T. gondii can infect and grow in any nucleated host cells which leading to the production of various inflammatory markers by the innate acute inflammatory responses and antigen-specific adaptive immunity which facilitates a state of chronic inflammation at various anatomical sites in the host [8]. In a meta-analysis of studies on the relation between chronic toxoplasmosis and diabetes mellitus, researchers found that chronic toxoplasmosis was a possible risk factor for type 2 diabetes mellitus [9].

Table (1) showed that a group of diabetic patients only has the highest level of fasting blood sugar as compared with other groups with highly significant differences.

Table 1. Levels of FBS in studied groups with their comparisons.

Groups	No.	Mean Pg/ml	Std. Dev.	Std. Error	Lower Value	Upper Value
Diabetic patients infected with toxoplasmosis	50	155.42	51.84	7.33	121	363
Diabetic patients	70	188.31	72.12	8.55	134	289.6
Control	50	111.41	10.48	1.94	75	110
LSD-Value	24.617**					
P-Value	0.0001					

The shown results similar to results of Modrek *et al.*[10] results 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 that found 131 diabetic patients had fasting blood sugar 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 ($P < 0.05$).

Several experimental evidences have been evaluated and suggested as plausible pathophysiological mechanisms to explain this relation, including:

1. Infected white blood cells assimilate improved migratory feature, causing the easier distribution of *Toxoplasma* in body organs, suchpancreas [11].
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 **[12].**
3. A probability, is that *T.gondii* itself may attack and destroy pancreatic cells directly, initiating pancreatitis and more importantly, diabetes **[11]**.
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 **[12].**
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***[13].**
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 **[13].**

While table (2) revealed that all samples of research have seronegative for anti-*Toxoplasma* IgM with highly significant differences as well as showed that 50 samples of diabetes have seropositive for anti-*Toxoplasma* IgG and 70 samples have seronegative for IgG Abs in addition, a group of diabetic patients infected with toxoplasmosis has highest levels of IgG Abs that compared with other groups with highly significant differences as clarified in table (3).

Table 2. Distribution of *T. gondii* infection according toToxo IgM & IgG in studied groups.

Diagnosis	Toxoplasmosis IgM/IgG	Diabetic Patients		Control		P-Value Sig.(*)
		No.	%	No.	%	
Flex reagent cartridge IgM	+ ve	0	0.00	0	0.00	1.00
	-ve	120	100	50	100	
Total		**120**		**50**		
Flex reagent cartridge IgG	+ ve	50	41.67	0	0.00	0.01
	-ve	70	58.33	50	100	
Total		**120**		**50**		

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

Diagnosis	Groups	No.	Mean IU/ml	Std. Dev.	Std. Error	Lower Value	Upper Value
Toxoplasmosis IgM	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					

Diagnosis	Groups	No.	Mean IU/ml	Std. Dev.	Std. Error	Lower Value	Upper Value
Toxoplasmosis IgG	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					

Lately, the immulite 2000 torch assay measure Toxo IgM and IgG in International Units per milliliter (IU/ml) of serum; this assay is simple, rapid and comparatively inexpensive needful 60–90 minutes for completion [14].

The previous results of IgM and IgG Abs matched with the research of El-Awady *et al.*[14] that include 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 and sernegative for IgM Ab. Althoughmatched with results of Shirbazou*et al.*[15]that clarified the prevalence of IgG and IgM Abs in diabetic patients were (56.6%) and (2.4%) as well as in control were (22.4%) and (1.6%) respectively, in additionmatched with results of Gokce*et al.*study [16]that include 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 returns discovered that the prevalence rate of IgG Ab was directly associated with duration of diabetes because of the weakened immune system of diabetic patients which also proposed that toxoplasmosispatients are more capable 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.
3. Decline stage (recession), in which nerve cells and β-cells destruction of more compensatory function reach to its limits [11].

The results of table (4) referred to serum levels of IL-33 in studied groups and explained that a group of diabetic patients infected with toxoplasmosis only has the highest level of IL-33 in comparison with other groups although significant differences showed in these results.

Table 4. Concentrations of IL-33 (pg/ml) in sera of studied groups.

Groups	No.	Mean IU/ml	Std. Dev.	Std. Error	Lower Value	Upper Value
Diabetic patients infected with toxoplasmosis	50	858.84	389.13	196.45	6.17	4553.6
Diabetic patients	50	556.67	112.75	248.82	24.25	2685.98
Control	25	315.58	61.80	58.54	152.69	821.24
LSD-Value	358.55*					
P-Value	0.0464					
Normal range	32 – 4000					

Table 5. Comparisons of IL-33 levels (pg/ml) estimated in sera of studied groups.

Parameter	Group 1	Group 2	Mean Diff.	P-Value	Sig. (*)
IL – 33 Concentration (Pg/ml)	Diabetes patients with toxoplasmosis	Diabetes patients	302.17	0.091	NS
		Control	543.26	0.0336	*
	Diabetes patients	Control	241.09	0.2501	NS

Interleukin-33 is a new member of the IL-1 superfamily of cytokines that is expressed by mainly stromal cells, such as epithelial and endothelial cells, and its expression is upregulated following pro-inflammatory stimulation[17]. It'sfunction both as a traditional cytokine and as a nuclear factor regulating gene transcription. It increases concentration in serum of *toxoplasmosis* (IgG) patients due to interleukins that regulate information transfer among different types of leukocytes during various stages of immune or inflammatory response[18]. This is assured by increase IgG, IgA and IgM, this return may indicate that autoimmune disease like *toxoplasmosis* might influence cytokine production in toxoplamosis patients[19]. The reduction in IL-33 levels in diabetic patients may due to the protective effect of IL-33 by reducing adiposity and improving glucose tolerance and insulin resistance, this interleukin strongly induces Th2 cytokine production from these cells and can promote the pathogenesis of Th2 related disease such as asthma, atopic dermatitis and anaphylaxis[18]. However, IL-33 has shown various protective effects in cardiovascular diseases such as atherosclerosis, obesity, diabetes type 2 and cardiac remodeling. Thus, the effects of IL-33 are either pro- or anti-inflammatory depending on the disease and the model[19].

Conclusion

This research has reached to the following conclusions:

1. Fifty samples of this research are diabetic patients type 2 infected with toxoplasmosis, this illustrate the relationship between diabetes and *T. gondii* which relates to depression of immune response of type 2 diabetic patients, that make them more susceptible to toxoplasmosis infection.
2. Diabetic type 2 patients infected with toxoplasmosis have the highest level of glucose in their blood as FBS test clarified.
3. Some of Diabetic type 2 patients infected have chronic toxoplasmosis infection (IgG antibodies appeared) while there is no appearance for acute toxoplasmosis (IgM antibodies).
4. Levels of IL-33 rise in diabetic type 2 patients infected with chronic toxoplasmosis.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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