Blood Lymphocytes Detection in Iraqi Diabetic Type 2 Patients Infected with Chronic Toxoplasmosis by Using Flow Cytometry

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Abstract

Toxoplasma gondii is an obligate parasite infects different species of mammals as intermediate hosts while infects number of family of cats as definitive hosts. It has three morphological types: tachyzoites, bradyzoites and sporozoites. It causes (H.I.) humoral immunity and (C.M.I) cell mediated immunity response which is measured one of the most characteristic immunological types of this parasite infestation. Diabetes mellitus (DM) is considered as a heterogeneous group of metabolic abnormalities with hyperglycemia due either to a reduction of insulin biological function or absolute insulin deficiency. Researchers discovered that long term infection with *T. gondii* may be dangerous agent for DM type 2, and there is no relation between toxoplasmosis and DM type 1. The aim of this study include detection of CD3 in Iraqi diabetic type 2 infected with chronic toxoplasmosis by using flow cytometry. This study included 100 blood samples were collected from Iraqi diabetic type 2 patients and 50 blood samples of healthy volunteers with age range (15-75) years and mean 49.9±12.8 from Al – Imam Ali general hospital during May 2018 until August in the same year. Diagnose of toxoplasmosis infection was done by using rapid diagnosis test/ cassette and by Toxo IgG antibodies immulite torch assay while diabetes diagnosis by fasting glucose tests in addition CD3 were detected by using flow cytometric method. Diabetic patients a group infected with toxoplasmosis have the highest level of blood blood sugar in comparison with other groups. Forty – five samples of diabetic type 2 infected with chronic toxoplasmosis by detection IgG antibody in highly level 106.2± 90.2 with highly significant differences in comparisons with other groups (diabetic patients 3.6± 1.2 and control group 4.0±0.6) while any results for IgM Abs didn't found in this study. Seventy – five samples studied in flow cytometry method which include twenty – five samples for each group. Diabetic patients with toxoplasmosis recorded highest CD3 its mean 30.7± 14.51 pg /ml flowed by diabetic patients group has 24.84±9.47 pg /ml and control group has 20.03± 9.42 pg /ml with significant differences.

Keywords: Chronic toxoplasmosis, Diabetes mellitus (type 2), Flow cytometry, CD3

Introduction

Toxoplasma gondii is an intracellular parasite that infects a spacious numerous of avian and several of mammals as intermediate hosts while infects number of family of cats as definitive hosts [(1)]. It has three morphological types: tachyzoites, bradyzoites (in tissues), and sporozoites. The infection with this parasite induce humoral and cell mediated immunity responses which is measured as one of the most distinctive immunological types of *T. gondii* infection, CMI is produced by the parasite, appearing in host protection against rapid growth of tachyzoite and resulting pathologic variations [(2)]. Transmission of *T. gondii* starts by ingestion of infected food or contaminated water containing cysts of the parasite; it can be transmissible through blood transfusion and organ transplant [(3)]. After infection, *T. gondii* moves to many organs of the host such as brain, lungs, heart, pancreas and most frequently in the lymph nodes [(4)]. The manifestation of toxoplasmosis in pancreas has been described in animals and humans. In man, the infection may be a reason to cause of pancreatitis [(5)].

Diabetes mellitus (DM) is signified as a heterogeneous group of metabolic abnormalities with hyperglycemia due either to a reduction of insulin biological function or absolute insulin deficiency. DM patients with long time are likely to develop multiple complications, such as neuropathy, atherosclerosis retinopathy and renopathy [6]. The National Diabetes Data Group of American Diabetes Association categorized DM into 2 major types according to the etiology in 1997: type 1 diabetes mellitus (T1DM) and T2DM. T2DM patients maintain their insulin secretion capability and their hyperglycemia can be regulated by oral anti-diabetic drugs [7].

It is unknown how pancreas involvement during toxoplasmosis may conduct to DM, In seroprevalence of toxoplasmosis among many diseases diabetes mellitus, neoplasms, arthritis etc , 1.265 patients were checked serum IgG antibody ,6.7% was positive seroprevalence for toxoplasmosis, this revealed that toxoplasmosis has a strong association with chronic infection like diabetes mellitus and neoplasms [8]. A meta-analysis research of a relationship between long term infection with *T. gondii* and diabetes mellitus, researchers suggestion that there was a risk reason between two diseases and carry out researchers for studies in this subject as well as no relationship between toxoplasmosis type 1 diabetes mellitus and toxoplasmosis [9].

The aim of this study includes detection of CD3 in Iraqi diabetic type 2 patients infected with toxoplasmosis by using flow cytometry.

Materials and Methods

Study samples

One hundred blood samples were collected from Iraqi diabetic type 2 patients and 50 blood samples of healthy volunteers with age range (15-75) years and mean 49.9±12.8 from Al – Imam Ali general hospital during May 2018 until August of the same year. Five milliliters of venous blood were collected using 5 ml disposable syringe. Two ml of blood sample was transferred to gel tube and left to get coagulation at (20-25°C) for 15 minutes then it centrifuged for 10 minutes at 2500-3000 rpm in order to separation serum that used for fasting blood sugar diagnosis (Glucose MR, Linear, Spain) then for *T. gondii* diagnosis by rapid diagnostic test/cassette (Tox IgM and/or IgG rapid test-casette, Paramedical, Italy) then by immulite torch assay (Flex reagent cartridge IgG, Siemens, Germany) and three ml was transferred immediately to EDTA-tube and left on blood role mixer for flow cytometry study (Flow cytometry, Partec, Germany). Fifty samples of diabetic patients with and without *Toxoplasma* infection and 25 samples of healthy volunteers were studied in flow cytometry study.

Flow cytometry

Seventy five samples were selected for flow cytometry by detection CD3 (Cy5PE-conjugated anti-CD3, Becton Dickinson and company BD Biosciences, USA) were performed the following steps:

1. Add the appropriate volume of CD3 flurochrome - conjugated monoclonal antibody to 100 µl of whole blood in a 12 x 75-mm capped polystyrene test tube, then vortex with caution and incubated for 15-30 minutes in the dark place at (20 - 25ºC).

2. Add 2 ml of brand flow cytometer lysing solution (BD FACS), then vortex the tube gently and incubate for 10 minutes in the dark at room temperature (20ºC - 25ºC).

3. Centrifuge the tubes at 300g for 5 minutes.

4. Aspirate the supernatant, and then added 2 ml of stain buffer solution consisting of PBS plus 0.2% bovine serum albumin BSA with 0.1% sodium azide.

5. Add 0.5 mL of stabilizing fixative, then vortex the tube gently and incubate the tube at (2 - 8ºC) in the dark room for 20-30 minutes.

6. Finally mix the tube thoroughly and then analyze the sample.

Statistical Analysis

Chi-square test and least significant differences (LSD) for significant differences were used to analyze statistically results of this study. P-value that used for statistical differences was ($P \leq 0.05$).

Results and Discussion

The existing hypothesis suggests that the incidence of toxoplasmosis rise the ability to infection with DM, and, in contrast, DM patients have more ability to

infection with are more able to infect with *T. gondii* [10].

Forty-seven samples of diabetic type 2 patients infected with toxoplasmosis as shown in table (1), all studied groups with their results in significant differences.

Table (1): Prevalence of toxoplasmosis depending on rapid diagnostic test/cassette in studied groups.

Diagnosis	Response	Diabetic Patients		Control		P-Value Sig.(*)
		No.	%	No.	%	
Tox IgG rapid diagnostic test/cassette	+ ve	47	43.8	0	0.00	0.0001**
	-ve	53	56.2	50	100	
Total		100		50		

Rapid diagnostic test (cassette) is a useful , lateral flow chromatographic immunotest for the simultaneous detection and differentiation of immunoglobulin-G and immunoglobulin-M Abs in sera and plasma specimens of human. This results agree with the result of Achonduh-Atijegbe *et al.,* [11] that studied the prevalence of malaria, typhoid, toxoplasmosis and rubella among febrile in 315 children with mean age 5.8 ± 3.8 years in Cameroon which found 10 (3.2%) children have recent infection with toxoplasmosis while 121 (38.3%) children have past infection. While table (2) showed that 45 samples of diabetic patients infected with chronic toxoplasmosis with high significant differences when comparison with other studied groups.

Table (2): Prevalence of toxoplasmosis based on Toxo IgG in different groups of the study.

Diagnosis	Toxoplasmosis IgG	Diabetic Patients		Control		P-Value Sig.(*)
		No.	%	No.	%	
Flex reagent cartridge IgG	+ ve	45	66.6	0	0.00	0.0001**
	-ve	55	33.4	50	100	
Total		100		50		

Also table (3) show comparisons of IgG levels in studied groups estimated by IU/ml. It was found that DM patients with toxoplasmosis has highest level of IgG 106.2 ± 13.1 followed by diabetic patients group has 3.6 ± 0.08 and healthy volunteers group has less level 4.0 ± 0.13 with highly significant differences $P \leq 0.0001$, any results for IgM Abs didn't found in this study.

Table (3): Different levels of IgG (IU/ml) in all studied groups .

Groups	No.	Mean IU/ml	Std. Dev.	Std. Error	Lower Value	Upper Value
DM patients infected with toxoplasmosis	45	106.2	90.2	13.1	14.2	255
Diabetic patients	55	3.6	1.2	0.08	2.4	5.1
Control	50	4.0	0.6	0.13	3.0	6.5
LSD-Value	22.38 **					
P-Value	0.0001					

In present time, torch assay 2000 immulite, an automated quantitative IgG and IgM for toxoplasmosis , It is revealed, that calculates Toxo IgG and IgM in International Units per milliliter (IU/ml) of serum. It has many features like easy, moderately inexpensive and fast requiring 60–90 min. for completion (12).

This results agree with results of El-Awady *et al.* [12] carried out a study on 110 pregnant women infected with for toxoplasmosis infected with diabetes as well as 110 healthy control (pregnant women) ,they get (42.7%) of diabetic pregnant women (47) were positive for IgG Abs and (21.81%) of healthy pregnant women (24) were positive for the same Abs . These answers clarified the pervasiveness percentage of IgG Abs has directly correlated with the diabetes duration due to the weakened immune system of DM patients as well as this study suggested that toxo-patients are more suggestible to be diabetics than those without it . Destruction of the pancreas happens in three steps of *Toxoplasma* infection:

1. Hyperactive stage (hyper-period) in which β-cell demolition of nerve cells and less intervention in the insect in a hyperactive state of the pancreas, occasionally the secretion of insulin is extreme, often resulting in low or a too low blood glucose, this stage is frequently happens during adolescence.

2. The disordered phase (compensatory stage), pancreatic β-cells and neurons have a great quantity of damage, under normal circumstances, the secretion of insulin will be deficient, the body will begin the compensative function. So, when few in the disordered state, this stage of insulin secretion eventually.

3. The stage of decline (depression), in which β-cells and nerve cells obliteration of more compensatory function achieve is confined [13].

Table (4) showed that a group of DM and toxoplasmosis has the highest level of blood sugar in significant differences when comparison with different studied groups.

Table (4): FBS levels in all studied groups with comparisons.

Groups	No.	Mean IU/ml	Std. Dev.	Std. Error	Lower Value	Upper Value
Diabetic patients infected with toxoplasmosis	45	155.2	52.3	7.1	110	365
Diabetic patients	55	150.2	50.4	6.4	115	300
Control	50	111.4	10.7	2.1	85	95
LSD-Value	24.6 **					
P-Value	0.0001					

Many mechanisms clarify the relationship between diabetes and toxoplasmosis such as:

1. Infected WBCs integrate improved migratory characteristic, cause of easy spread of *Toxoplasma* parasite to body organs, including pancreas [14].

2. Establishment of nitric oxide (NO) and reactive oxygen species (ROS) are motivated via DM, and these factors , as motivating of intracellular pathogens, that can reactivate latent, parasites cysts, over starting acute infection.

3. Given the neutrophils failure to correctly achieve phagocytosis and intracellular killing in advanced phase of DM, there may be expand in responsiveness to intracellular pathogens such as *Toxoplasma* and *Candida* [15].

Flow cytometry study included 25 samples for each previous groups which showed in table (5) a diabetic with toxoplasmosis patients group has the highest CD3 mean 30.7 ± 14.51 pg/ml with value ranging from 13.1 to 42.3 while a group of diabetic patients only has 24.84 ± 9.47 pg/ml ranging from 6.2 to 28.46 in comparison healthy control group 20.03 ± 9.42 pg/ml ranging from 2.9 to 33.3 with significant differences showed in these results ($P \leq 0.05$).

Table (5): Statistical description of CD3 in blood of studied groups.

Groups	No.	Mean IU/ml	Std. Dev.	Std. Error	Lower Value	Upper Value
Diabetic patients infected with toxoplasmosis	25	30.7	14.51	4.5	13.1	42.3
Diabetic patients	25	24.84	9.47	3.82	6.2	28.46
Control	25	20.03	9.42	3.95	2.9	33.3
LSD-Value	8.642 **					
P-Value	0.05					

CD3 (cluster of differentiation 3) was recognized by Kung and colleagues in 1979 and was one of the first groups of human T lymphocyte surface antigens recognized using monoclonal antibodies, CD3 T cell co-receptor helps to stimulate both the cytotoxic T cell (CD8+ naive T cells) and also T helper cells (CD4+ naive T cells), it comprises of a protein complex and is combined of four distinct chains. In mammals, the complex includes a CD3γ chain, a CD3δ chain, and two CD3ε chains, these chains relate with the T-cell receptor (TCR) and the ζ-chain (zeta-chain) to cause stimulation signal in T lymphocytes, the TCR, ζ-chain, and CD3 molecules together create the TCR complex [(16)]. It was successively shown that antibodies against CD3, contingent on the circumstances used, either activate T cells to divide or reduce the development of effector functions like cytotoxicity, so it was obvious early on that CD3 had an important part in T cell function, it is originally articulated in the cytoplasm of pro-thymocytes, the stem cells from which T-cells elavated in the thymus, This great specificity, joined with the attendance of CD3 at all stages of T-cell development, makes it a useful immunohistochemical marker for T-cells in tissue portions, Ag stays existing in almost all T-cell lymphomas and leukaemias, and so it can be used to differentiate them from superficially like B-cell and myeloid neoplasms, figure (1). The conclusion of this study , the infection with *T.gondii* induced the immunity responses (cellular and humoral) , also toxoplasmosis elicit response of T cells in acute response CD4+ cells as well as in chronic infection CD8+.

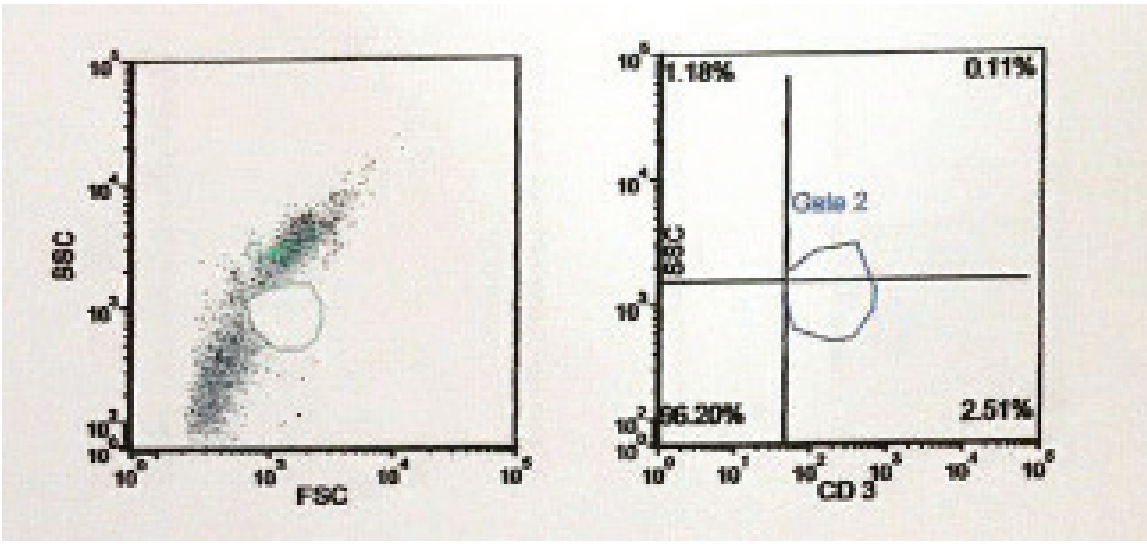

Fig. (1):- Flow cytometric curve show CD3 in diabetic patient type 2 not infected with toxoplasmosis.

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