



THE ROLE OF SOME CYTOKINES IN DIABETIC PATIENTS INFECTED WITH TOXOPLASMOSIS

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Abstract

Toxoplasmosis is the term for infection and disease in man and animal caused by a parasite called *Toxoplasma gondii*. The more susceptible to infect with toxoplasmosis is the Diabetic patients, due to low level of immunity response. The aim of current study is to investigate the immune status of diabetes mellitus type 2. One hundred and seventy five samples of both diabetes mellitus type 2 patients and controls which had been tested by ELISA technique to detect anti-*Toxoplasma* Abs (IgG and IgM). The positive toxoplasmosis samples were tested to detect the level of TNF alpha and MIG. Results for all samples clarified that seronegative for IgM antibodies while 53 (53%) diabetic patients were seropositive for IgG antibodies and for toxoplasmosis only patients were 30 (40%) and 45 healthy as a (controls) were seronegative for IgG antibodies and diabetic with highly significant differences (P. value \leq 0.0001). Serum level of TNF alpha was recorded an increase in a group of diabetic with toxoplasmosis patients (84.93 ± 18.33 pg/ml) with highly differences of significant (P < 0.004) also MIG level was increased in a group of *Toxoplasma gondii* only (14.439 ± 18.930 pg/ml) with no significant differences.

Key words: Toxoplasmosis, MIG, TNFalpha, diabetes mellitus type 2.

Introduction

Toxoplasma gondii is an obligate intracellular protozoan and mammalian host range belongs to phylum Apicomplexa, and depend its feed on humans and other warm blooded animals (Al-Shammaa, 2014). The disease which is caused by *Toxoplasma gondii* and a common disease occurrence and widespread prevalence in population groups is Toxoplasmosis. About (1/3) of the world's population is with toxoplasmosis infection (Robert-Gangneux, *et al.*, 2017). In warm-blooded vertebrate hosts the *T. gondii* represent as an intermediate hosts only and can pass by both asexual and sexual parts of the cycle life in Felidae (cats) that is act as both final and intermediate host they are the only one able to lay oocysts through contaminated feces. Tissue cyst and sporulated oocyst are the important sources of human infection through intake of undercooked meat or food and water contaminated (Robert-Gangneux and Dardé, 2012). Or tachyzoite across the placenta (placental transmission) from infected mother to embryo which caused abortion or birth fetal disorders (hydrocephalus, retinochoroiditis) (Vimercati *et al.*, 2017). Toxoplasmosis infection can be very serious, severe or life-threatening and even fatal in some individuals like fetuses, neonates, immunocompetent person and in immunocompromised patients (cancer, AIDS and other diseases). Can be diagnosed by different techniques by ELISA, Latex agglutination test and PCR (Sinjin *et al.*, 2004).

When *T. gondii* inter inside body the stimulation for immune response (humoral) which initiation for produce of antibody (IgG, IgM, IgE and IgA) from B-cell against the parasite and excretion, Cellular immunity which shearing specially Macrophage and Natural killer cell and Neutrophils. Cytokines plays an important role in immune response which describe as small molecule the nature of proteinuria or sugary proteins which are excreted by many body cells and white blood cells have molecular weights ranging from 10 to 23 Dalton.

Diabetic Mellitus it's chronic and one of an autoimmune system disease which distinguished by persistent hyperglycemia with disorder of protein, carbohydrate and fat-metabolism which is the result of abnormalities in insulin action or secretion or both. Diabetes disease is an factor rise the ability and risk of many infections in the host due to decrease of the immune system such disease *T. gondii* which act as an opportunistic infections appear through decrease of the immune system of patients with diabetes or other disease. As its presence in the pancreas can directly weaken or destroy of the pancreatic cells. When cells of β are destroyed, secretion of insulin would be influenced which increase the danger of diabetes. The diabetes has been classified into four clinical classes by American Diabetes Association (ADA): Type one (insulin-dependent diabetes mellitus), Type two (non-insulin-dependent diabetes mellitus), Type three Pregnant women can also get diabetes, called gestational diabetes and Type four it is caused by diseases of the exocrine pancreas like cystic fibrosis or drugs or chemicals, or after transplantation of organ insulin action or specific genetic defects of β -cell function.

The cytokine is an parts of the disease-defense system like in all aspects of adaptive and innate immune responses, involve differentiation and cellular growth, repair inflammation and cellular growth, but found to play a wider function in the (body structure /related to the study the functions of body). And have a small molecular-weight soluble protein.

Tumor Necrosis Factor (TNF) is a polypeptide protein which initially manufacture as a pro peptide by an enzyme TNF- α converting enzyme (TACE) to turn into a full form secretary TNF-alpha consist from 157 amino acids (Yap *et al.*, 1998), mainly produced from activation of Macrophage, dendritic cell and T-Lymphocyte and less producing from B-Lymphocyte at *T. gondii* infection.

Studies have showed that Tumor Necrosis Factor alphahave an essential role in the innate immunity against *Toxoplasma gondii* and effect the adaptive immune response.

And it is essential immunomodulators that act throughout the beginning of infection.

Development of a Th1-type immune response by the host is the major mechanism in the removal of intracellular parasites. The major cells responsible for parasite decision in this response are dendritic cells, macrophages and polymorphonuclear neutrophils which produce cytokines like IL-12 and TNF- α that motivate NK (Natural Killer) cells to produce INF- γ . Tumor Necrosis Factor alpha is created by T lymphocytes, mast cells and monocytes/macrophages and catalyze the micro-bicidal action of these cells, influential synergistically against *Toxoplasma gondii* (Hunter *et al.*, 1995).

Chemokine is low molecular weight & which is a super family of cytokines. chemokines have four classes of which are CC, CXC, CX3C, & XC. Which spend biological effects from G-protein coupled receptor. When connect to an appropriate receptor, the chemokine initiate signaling cascades which result in extensive variety of responses, like polarization, gene expression, cellular adhesion, chemotaxis and often receptor internalization and contributing to a protective immune response. Also its major function leads to immigration of cells of receptor to the chemokine position secretion as chemoattractants.

(MIG) or (CXCL9) is Monokine induced by interferon gamma and it's a part of INF- γ inducible sub-set of CXC chemokines, which plays an important function in host defense (Zychowska *et al.*, 2015) and (Lau *et al.*, 2000).

The aim of current study is to determine the levels of cytokine TNF-alpha and chemokine MIG in sera also it is designed to determine the effect of the infection with chronic toxoplasmosis may aid in a better understanding of the immunological mechanisms that can lead to chronic toxoplasmosis in Iraqi diabetic and non diabetic women.

Material and Methods

Hundred diabetic and nondiabetic women suffering from toxoplasmosis to department of National Center for Diabetes in Al-Yarmouk hospital and Private laboratories in Baghdad city from One December 2018 till Thirty of April 2019 were conducted in this study. The persons' ages range from (20–79) years, in addition a group of control were 75 of women.

From each woman (5 mL) of venous blood samples were collected and placed in test tube where the serum leaves it for one hour at room temperature to allow the clot to form. and then the serum separated by a centrifugal device of 3000 rpm for 15 min. which divided in eppendorf tubes (200 μ l) and preserved at deep freeze (-20 °C) till it is used. When sampling has been completed, the level of glucose Fasting Blood Sugar (FBS) has been measured using the enzymatic technique. In this technique, serum and one ml of selected solution has been mixed and brooded for 10 min. at 37 degree centigrade. The results were obtained by using Spectrophotometer device at wavelength 450 nm.

The (IgG, IgM) antibody level against *T. gondii* was read through ELISA technique using the available kits (Foresight kit) which prepared according to company manufacture by using ELISA techniques. In order to differentiate between positive and negative samples, the obtained value of cut off has been compared with the following ratios:

Positive sample: Cutoff < 11.0 IU/mL.

Negative sample: Cutoff < 0.9

Serum Level of Cytokine and Chemokine:

Sera of pregnant women that have a positive IgM antibody against *T. gondii* and control group were enrolled to measure levels of cytokine TNF alpha and chemokine MIG by using ELISA test. All kits were provided from Abcam; USA. The manufacturer's protocols has been followed for each kit and recombinant reference cytokine and chemokine samples has been served as positive control for calibration.

Data Analysis

The program of statistical analysis system that was used is SPSS version 25 to analyze the results of present study. Mean and SD has been used to determine the significant differences as probability. The confidence level is 95% and P-value is less than 0.05 which is the level of significance. ANOVA test is used to comparisons between the groups.

Results

Sera for 175 samples have been analyzed. Table 1 shows the distribution of (IgG/ IgM-Abs) in both healthy controls, diabetic with toxoplasmosis infection women patients, (53 %) samples from 100 type 2 diabetic patients have positive response for *Toxo* IgG in ELISA test have diabetes with toxoplasmosis, while (47%) diabetic only have negative response for ELISA IgG which have diabetes only, and 30 (40%) women suspected infection with *Toxoplasma gondii*. While IgM antibody did not appear. Such difference was highly significant between these groups ($P \leq 0.0001$).

Table 1 : distribution of *T. gondii* and Diabetes in controls and diabetes and Toxoplasmosis

Group	No. of tested samples	No. (+) for toxoplasmosis (%)
Diabetes patient	100	53 (53%)
Healthy controls	75	45 (100%)
P. value	0.000	**

Table 2 shows the distribution of toxoplasmosis according to the age of study group, considering the age groups and it's relation with the infection diabetes and toxoplasmosis disease.

The results showed that the highest prevalence was between (40-49) years of diabetic patients infect with toxoplasmosis which was 17 (32.1%), followed by group among (50-59) years, while the lowest percent was 1 (1.9%) in age group among 20-29 years.

Table 2 : Prevalence of Toxoplasmosis and diabetes with the age groups

Age year	No. Diabetes with toxoplasmosis %	No. Diabetes %
20 – 29	1 (1.9%)	1 (2.13%)
30 – 39	2 (3.8 %)	2 (4.3%)
40 – 49	17 (32.1 %)	7 (14.9%)
50 -59	14 (26.4 %)	11 (23.4%)
60 -69	11 (20.8 %)	12 (25.5%)
70 -79	8 (15.1 %)	14 (29.79%)

Results presented that 53 patients have FBS (Fasting Blood Sugar) levels were 200 to 300 mg/dL which has Toxoplasmosis of 26 (49.06%) as shown in Table3.

Table 3 : The Level of Glucose (FBS) and *T. gondii* Infection

Level of Glucose mg/dl	Toxoplasmosis IgG+	%
100-200	16	30.19
200-300	26	49.06
300-400	11	20.75
Total	53	100

The results of cytokines level shown in Table (4) describe that a significant rise in cytokine TNF alpha comparing with production in the control group in Toxoplasmosis with diabetes (84.93 ± 18.33 pg/ml), then mean value for diabetic only was (78.25 ± 24.78 pg/ml) and then lowest one was for *Toxoplasma gondii* (66.73 ± 18.67 pg/ml).

Table 4 : Descriptive Statistics for All Groups for TNF alpha.

Descriptive Statistics					
group	No.	Min.	Max.	Mean	Std. Deviation
<i>T. gondii</i> with Diabetic 2	25	43.484	130.932	84.936	18.335
Diabetic 2	24	28.854	169.614	78.252	24.782
<i>T. gondii</i>	21	29.475	100.011	66.737	18.671
Control	20	20.475	122.511	63.473	28.517

The results of chemokine level show in table (5) there was a significant decrease in chemokine MIG compared to production in the control group in Toxoplasmosis with diabetes (3.925 ± 9.747 pg/ml), then mean value for diabetic only was (5.11 ± 7.116 pg/ml) and then highest one was for *Toxoplasma gondii* (14.439 ± 18.930 pg/ml).

Table 5 : Descriptive Statistics for All Groups of MIG.

Descriptive Statistics					
Groups	N	Mini	Max	Mean	Std. Deviation
<i>Toxoplasma</i> with Diabetic	24	0.890	46.534	3.925	9.747
Diabetic	24	0.890	25.952	5.110	7.116
<i>Toxoplasma gondii</i>	22	0.890	59.958	14.439	18.930
Control	20	0.890	26.847	10.021	8.567
Valid N (list wise)	20				

Discussion

High distribution rates of D.M. and toxoplasmosis in Iraq due to many factors like genetically or environment conditions and numerous nations around the whole world. Also keeping in mind the essential role of the complications and impairment of the cellular and humoral immunity resulted from D.M., the present study aimed to determine the levels of sera of *Toxoplasma* antibodies in diabetic persons and measured the levels of both some cytokine and chemokine (TNF alpha and MIG).

The results showed that 100 cases among 175 diabetic patients had seropositive for IgG. The results were agreed with the study by Gokce that Anti- *T. gondii* antibodies in type 2 diabetes the results was 56.6% seropositive for IgG. (Sharad and Al-Hamairy) have a seroepidemiological study in order to examine the seroprevalence of *Toxoplasma gondii* in diabetic person in Babylon Province, Iraq their results was

showed that the seropositivity rates were 51.4% for the *T. gondii* IgG antibodies as detect by ELISA test.

While did not agreed with Shirbazou and followers appeared that sixty percent of diabetic patients had specified IgG antibody *Toxoplasma gondii*. And the study reported by (Hamad, H.K.) 2017 in Iraq IgG was 82.1%

There are many factors like diabetes period, noninfectious complications severity, miscarriage, ocular disease, comorbidity, co-infection and meat consuming that could lead to chronic and acute of patients suffer from diabetic infection as well as severe disease development. The highest distribution ratio of *Toxoplasma gondii* IgG and IgM anti-bodies has been noticed in the old age women.

The current study results showed that median value of IgM- antibodies of *Toxoplasma* in women with or without diabetes and with toxoplasmosis was 0, this result reflects that infected women had chronic toxoplasmosis, while for IgM antibodies was less than cut-off value which might be referred to the infection nature in which most of the women infected didn't attended the hospital through the acute stage until miscarriage happens or have diabetic disease, the life span for IgM less than 14 days.

The results of a current study has been compared with the results of (Shirbazou *et al.*, 2013) in Iran which has detection of serologic of anti *T. gondii* infection in 55 level FBS of diabetic patients that find the category of diabetic patient has glucose levels of (200-300) mg/dl.

Results of this study agreed with (Spranger *et al.*, 2003) which tested the effects of TNF-alpha on the diabetes mellitus type 2 development and supervising high IL-6 and TNF-alpha levels in persons with Type 2 Diabetes Mellitus.

The causing of the increasing to the important function of TNF alpha in defense against the infection of parasite inside the cells, which play with the other cytokines to proliferation and differentiation of B- cell.

TNF- α is a Th-I response cytokine created by monocytes, macrophage, T-lymphocyte, and basophils. And is a multifunctional cytokine produced by a wide domain of cell populations.

The present study seen that there was no significant increased of MIG in the sera of Toxoplasmosis infected Diabetic persons compared with control group, this lowering was the result of the continuation of the stimulus antigens parasite, leading thus to inhibition of the activation of immune cells manufacture MIG leading to low level in the sera of patient compared to serum of the control groups, while increasing the level of MIG in the serum of group of *T. gondii* patients compared to control, may be due to the MIG is critical of T cells recruitment of in the immune areas that breed tachyzoites to prohibit the chronic activate *T. gondii* infection (Khan 2000).

Conclusions

The levels of cytokine (TNF- α) was highly in diabetic with toxoplasmosis patients more than other groups which was affected in this disease which are evidence of the role Th1 & Th2 in chronic infections, while chemokine (MIG) increase in a *T. gondii* infection groups in the present study includes a chronic infections of *T. gondii*. According the present result there was a relationship between Diabetes with toxoplasmosis that related with the inhibition of immune

response of the diabetic patients made them more suitable to toxoplasmosis infection.

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