# Evaluation of Soluble PD-1 and PD-L1 in Iraqi Type 2 Diabetes Mellitus (T2DM) Patients with Chronic Toxoplasmosis

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## Abstract

*Toxoplasma gondii* is an important opportunistic parasite in immunocompromised people. Globally, diabetes mellitus is one of the most challenging public health burdens of the 21st century. Both toxoplasmosis and diabetes are very common in Iraq and other countries. The main objective of the present study was to investigate the sero- prevalence of the anti-*Toxoplasma gondii* IgG antibodies in Iraqi T2DM patients and to clarify the role of soluble programmed death-1 (sPD-1) and ( sPD-L1) in Iraqi T2DM patients with chronic toxoplasmosis. Enzyme Linked Immunosorbent Assay (ELISA) was used to detect anti- *T. gondii* IgG antibodies in the sera of 180 patients with type 2 diabetes mellitus (T2DM) and 163 apparently healthy controls. The results showed that 117(34%) samples of sera patients have been founded T2DM with toxoplasmosis, 63(18%) samples have T2DM, 55(16%) cases have control toxoplasmosis (those patients were had toxoplasmosis but showing no symptoms) and 108 (32%) cases samples were considered as a control group without any infections. Sera (sPD-1 and sPDL-1) levels were determined by ELISA using a quantitative sandwich enzyme immunoassay technique. The results showed that levels of sPD-1 and sPDL-1 levels were significantly higher in patients group than healthy subjects (P<0.01).

***Keywords:*** *Toxoplasma gondii, chronic toxoplasmosis, T2DM, sPD-1 and sPDL-1*

## Introduction

*Toxoplasma gondii* (*T. gondii*) is an intracellular obligate parasite that belongs to phylum Apicomplexa which causes Toxoplasmosis [(1)]. Cats and other Felidae family members implicate as a definitive host of the parasite. Humans and other livestock are considered as intermediate host of *T. gondii* [(2)].

Toxoplasmosis is usually asymptomatic in infected humans. However, some infected group such as immunocompromised patients, suffer from fatal and life-threatening outcome including encephalitis, pneumonia, retino-choroiditis, epilepsy and other neurological complication [(3)].

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Toxoplasmosis is implicated in numerous autoimmune diseases for example rheumatoid arthritis, polymyositis, thyroid autoimmunity and anti-phospholipid syndrome [(4)]. However, *T. gondii* infection in individuals with type 2 diabetes mellitus (T2DM) has received little recognition.

The T2DM is the most common type which accounts for about 90% of all diagnosed diabetes cases [(5)]. The disease reduces cellular and humeral immune status through their deplorable long hyperglycemic course and possibly stimulates latent opportunistic pathogens [(6)]. The prevalence of diabetes is reaching epidemic levels and it is estimated that 642 million people will be living with diabetes by 2040, up from 415 million in 2015. Toxoplasmosis is a disease caused by the protozoan parasite *Toxoplasma gondii*. Up to one third of the world's human population is estimated to be carrying *Toxoplasma* infection and the life cycle of this parasite plays a huge role in its easy transmission [(5)].

Both toxoplasmosis and T2DM are very common in Iraq and other countries [7].

Programmed death-1 (PD-1) is a novel member of the CD28 superfamily. It's a negative co-stimulatory factor that mediates negative co-stimulatory signal [8]. PD-1 can effectively inhibit functioning and proliferation of the T cells, and reduce IL-2, IL-10 and IFN-γ secretion [9] playing an important role in immune regulation [10].

In recent years, more and more studies indicate that the pathogenesis of T2DM involves immune dysfunction, and T2DM can be considered somewhat a chronic inflammatory disease [11]. Impaired immunity in patients with diabetes may increase the risk of infection; however, the exact effect of diabetes on patients with toxoplasmosis remains to be studied. The main objective of the present study was to investigate the sero- prevalence of the anti-*Toxoplasma gondii* IgG antibodies in Iraqi T2DM patients and to clarify the role of soluble programmed death-1 (sPD-1) and ( sPD-L1) in Iraqi T2DM patients with chronic toxoplasmosis.

## Materials and Method

### Subjects and Samples

This study was included 180 samples of patients with Type 2 diabetes mellitus (T2DM ) attending to Department of diabetes in Al-Iamamain Al-Kadhumain Teaching Hospital in Baghdad ,Iraq. During the period from December 2018 to April 2019. Out of this sample, a group of 163 healthy subjects were considered as control group. The age of all patients and healthy subjects were ranged from 39 – 79. Five ml of venous blood were collected from each subjects (patients and control) and placed in gel tube, the serum was separated and divided in ependorff tubes then stored at -20C° until it is used.

### Serological tests

**1- ELISA *T. gondi* – IgG :** The sera of all samples (Patients and control) were tested with the presence of specific IgG antibodies of *Toxoplasma gondii*, via ELISA kits which had supported by (Bioactiva Company, Germany) and applied the test according to the manufacturer's instructions.

**2-Serum Level of PD-1:** Serum levels of **PD-1** was measured by using specific enzyme-linked immunosorbent assay) ELISA) kit (R&D Company, USA), according to the manufactures protocol.

**3-Serum Level of PD-L1:** Serum levels of **PD-L1** was measured by using specific enzyme-linked immunosorbent assay) ELISA) kit (Sunlong Biotech Company, China), according to the manufactures protocol.

### Statistical Analysis

The Statistical analyses were done by Statistical Package for the Social Sciences for Science (SPSS) version 2010. The statistical tests was included Descriptive statistical tables, Mean, Standard Error, Standard Deviation, 95% Confidence Interval of the Mean under $P > 0.05$ and $P < 0.01$ to considered statistically significant.

## Results

In the summarize examine results, the study samples showed that 117(34%) samples of sera patients have been founded T2DM with toxoplasmosis , 63(18%) samples have T2DM, 55(16%) cases have control toxoplasmosis (those patients were had toxoplasmosis but showing no symptoms) and 108 (32%) cases samples were considered as a control group without any infections (Table1).

The cut– off value of positive IgG (10 IU/ml) in all studied groups. The results recorded in the table 1 were shown higher results of levels of IgG in T2DM with toxoplasmosis group as 22.65± 8.55 IU/ml, followed by positive control group 19.59±7.52 IU/ml, and T2DM group with value 5.98±2.09 IU/ml, while negative control group presented low results of this antibody 3.48±2.40 IU/ml.

**Table 1: Levels of IgG antibodies (IU/ml) for all study groups.**

| Groups | No. of Samples | % | Mean ± SD. | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| Type 2 diabetes mellitus (T2DM) with Toxoplasmosis | 117/180 | 65 | 22.9 ± 6.42 | 21.71 | 24.06 |
| T2DM | 63/180 | 35 | 1.51 ± 5.5 | 5.17 | 5.93 |
| Positive control | 55/163 | 16 | 8.73 14.3 ± | 11.90 | 16.62 |
| Negative control | 108/163 | 32 | 3.0 ±1.98 | 2.64 | 3.39 |

Table (2) shows the comparisons in the means of the IgG among all studied groups, highly significant differences ($P < 0.01$) were registered when comparing the values of IgG for the patient's in T2DM with toxoplasmosis and T2DM only, positive control and negative control .

**Table 2: Multiple comparisons of the IgG concentrations ( IU/ml) according to**

| Parameter | Group(1) | Group(j) | P-value | Sig. |
|---|---|---|---|---|
| IgG | Type 2 diabetes mellitus (T2DM) with Toxoplasmosis | T2DM | 0.000 | HS |
| | | Positive control | 0.000 | HS |
| | | Negative control | 0.000 | HS |
| | T2DM | Positive control | 0.000 | HS |
| | | Negative control | 0.000 | HS |
| | Positive control | Negative control | 0.000 | HS |

HS: Highly Significant at P< 0.01

Games-Howell (G.H.) test for potential couples between studied groups

Table (3) referred to the differences of the means for sPD-1 among all studied groups, the results didn't record significant difference when comparing the level of sPD-1 in T2DM patients with toxoplasmosis and the groups of T2DM, positive control respectively, while high significant differences at probability of $P<0.01$ were recorded when comparing the negative group.

**Table 3: Multiple comparisons of the sPD-1 concentrations (pg/ml) according to**

| Parameter | Group(1) | Group(j) | P-value | Sig. |
|---|---|---|---|---|
| sPD-1 | Type 2 diabetes mellitus (T2DM) with Toxoplasmosis | T2DM | 0.817 | NS |
| | | Positive control | 0.088 | NS |
| | | Negative control | 0.000 | HS |
| | T2DM | Positive control | 0.241 | NS |
| | | Negative control | 0.000 | HS |
| | Positive control | Negative control | 0.068 | NS |

HS: Highly Significant at P< 0.01; NS: No Significant at P> 0.05

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**Table (4) showed high level of sPDL-1 in the group of T2DM with toxoplasmosis patients 127.5 ±29.31 pg/ml compared to T2DM 114.2 ±17.89 pg/ml, positive and negative control 123.4 ±34.53 pg/ml 116.3±24.44 pg/ml respectively.**

**Table 4 : Levels of sPDL-1 (pg/ml) for all study groups.**

| **Groups** | **No. of Samples** | **Mean± SD** | **95% Confidence Interval for Mean** | |
|---|---|---|---|---|
| | | | **Lower Bound** | **Upper Bound** |
| Type 2 diabetes mellitus (T2DM) with Toxoplasmosis | 60 | 127.5 ±29.31 | 119.9 | 135.0 |
| T2DM | 31 | 114.2 ±17.89 | 111.3 | 117.1 |
| Positive control | 16 | 123.4 ±34.53 | 105.0 | 141.8 |
| Negative control | 69 | 116.3 ±24.44 | 110.4 | 122.1 |

**Table 5: Multiple comparisons of the sPDL-1 concentrations (pg/ml) according to**

| Parameter | Group(1) | Group(j) | P-value | Sig. |
|---|---|---|---|---|
| sPDL-1 | Type 2 diabetes mellitus (T2DM) with Toxoplasmosis | T2DM | 0.008 | HS |
| | | Positive control | 0.972 | NS |
| | | Negative control | 0.096 | NS |
| | T2DM | Positive control | 0.722 | NS |
| | | Negative control | 0.920 | NS |
| | Positive control | Negative control | 0.862 | NS |

HS: Highly Significant at P< 0.01; NS: No Significant at P> 0.05

Games-Howell (G.H.) test for potential couples between studied groups

## Discussion

The main goal of the current study was to investigate the sero prevalence of the anti-*Toxoplasma gondii* IgG antibodies in Iraqi T2DM patients and to clarify the role of soluble programmed death-1 (sPD-1) and (sPD-L1) in Iraqi T2DM patients with chronic toxoplasmosis. The results of the present study revealed that 65% of the diabetic subjects were found seropositive for the anti-*T. gondii* IgG antibodies while 35% of the non-diabetic individuals were found seropositive with the same antibody.

Consequently, the risk factor for *T. gondii* infection in diabetic patients was about two folds higher than in healthy controls. Therefore, patients infected with *T. gondii* may be more at risk to develop diabetic than uninfected individuals.[10]. Recently, Sharad and Al-Hamairy[12] conducted a sero epidemiological study in order to detect the sero prevalence of *T. gondii* in diabetic individual in Babylon Province, Iraq and their results showed that the sero positivity rates were 51.4% for the *T. gondii* IgG by ELISA test. Siyadatpanah et al. [13] investigated the anti-*T. gondii* IgG antibodies in diabetic and non-diabetic individuals in west Mazandaran province, Iran, using ELISA method and their results showed that 52.6% of the diabetic individuals were seropositive for anti- *T. gondii* IgG while 50.6% of the non-diabetic individuals were seropositive for the same antibody. Recently, Modrek et al. [14] conducted a study to estimate the serum levels of *T. gondii* IgG antibodies in 205 diabetic patients in Iran and their results showed that the overall sero positivity rate was 70.7%. Molan and Ismael [7] detected a study to determine the serum levels of *T. gondii* IgG antibodies in 300 T2DM patients in Diyala Province, Iraq and their results showed that the overall sero positivity rate was 66%.

Although toxoplasmosis and diabetes may pave the way to each 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. Previous studies have confirmed the occurrence of necrotic lesions and inflammation in various organs (pancreas, stomach, lymph nodes and intestine) of the experimental animals infected with *T. gondii* and the tachyzoites have been detected in these lesions [13,14].

Oz [15] reported that *T. gondii* infects nucleated cells including pancreatic and may destroy the β cells and secretion of insulin and increase the risk of acute and chronic pancreatitis as well as diabetes. In addition, the tissue necrosis in pancreas during acute toxoplasmosis has also been reported [14]. The bradyzoites of *T. gondii* have been detected inside tissue cysts in the pancreatic tissues, acinar cells and bile duct epithelial cells [16].

Moreover, Prandota et al. [17] reported that *T. gondii* plays an important role in the pathogenesis of both types of diabetes. Consequently, during the process of organ transplantation, the recipients of pancreas and kidney containing the tissue cysts that contain the bradyzoites, are at a great risk for toxoplasmosis due to immunosuppressive chemotherapy and/or reactivation of bradyzoites [18].In contrast, T2DM has been considered as chronic inflammatory disease that induces various changes to immune cell function[19].

Programmed death-1 (PD-1) is a novel member of the CD28 superfamily. It's a negative co-stimulatory factor that mediates negative co-stimulatory signal [8]. PD-1 can effectively inhibit functioning and proliferation of the T cells, and reduce IL-2, IL-10 and IFN-γ secretion [9] playing an important role in immune regulation [10].

In this regard, there are no available literatures about the role of sPD-1 and sPDl-1 in toxoplasmosis. However, some studies have evaluated the serum level of sPD-1 during other infectious disease. These studies observed significantly higher levels of sPD-1 among patients with chronic HCV [20], *Echinococcus granulosus* [21], pulmonary tuberculosis and those whom having active pulmonary TB with co-incidental *Strongyloides stercoralis* [22] infection than control subjects free from these diseases.

Thus, increase in sPD-1 level inhibits the PD-1/ PD-L1 signaling pathway in T cells through negative feedback. Consequently, it reduces the inhibition of T cell activation and increases the activity of the immune system for managing CL. This supports the findings of Wang *et al*. [23] who suggested that sPD-1 blocks the membrane PD-1 binding site on activated T-cells, thereby attenuating the PD-1 signaling pathway and increasing the immune response.

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

**Conflict of Interest**: Non

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