

Seroepidemiology of *Toxoplasma gondii* in diabetic patients type 2 by enzyme-linked immunosorbent assay method in Zabol City, 2017–2018

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Background: Type 2 diabetes mellitus (T2DM) is prone to opportunistic infections, including toxoplasmosis, due to an immunodeficiency system. This study aimed to evaluate the serum of people with T2DM to determine the titer of anti-toxoplasma antibodies in patients and compare it with the control group. **Materials and Methods:** 720 blood samples have been carried out between October and the end of January 2017 in Sistan, and Baluchestan provinces in southeastern Iran, of these, 360 samples were related to healthy individuals (control), and 360 samples were related to T2DM individuals. The immunoglobulin (Ig) M and IgG enzyme-linked immunosorbent assay methods have been used to detect toxoplasmosis. The data were analyzed using SPSS-19, Chi-square, and Fisher's exact test to compare statistical parameters. **Results:** In this cross-sectional study, out of 360 samples of T2DM by ELISA method, 60% samples in diabetic patients and 48.1% in control group were IgG positive ($P < 0.05$). Nearly 2.5% samples in diabetic patients and 0.3% in control group were IgM positive ($P < 0.05$). **Conclusion:** Anti-toxoplasma antibodies including IgG and IgM were higher in diabetic patient in comparison to control group.

Key words: Antibody, diabetes, enzyme-linked immunosorbent assay, *Toxoplasma gondii*

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INTRODUCTION

Diabetes is a relatively common metabolic disorder in the world, manifested by an increase in blood glucose levels over some time.

Diabetes falls into two general categories

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder in which genetic, environmental, and immunological factors determine the selective degradation of insulin-secreting beta cells in the pancreas.^[1] Type 2 diabetes mellitus (T2DM) is a group of metabolic diseases that occur when a person has high blood sugar levels and the pancreas is unable to produce enough insulin, or the cells respond to the

insulin produced.^[1] With the decline in physical activity and changes in diet and lifestyle, its prevalence has increased dramatically.^[2] This epidemic has created major health problems for human societies. Diabetes is the fifth leading cause of death in Western societies and the ninth leading cause of death in Iranian men.^[1] This number indicates that there are 415 million people with diabetes in the world in 2015, and this number will increase to 642 million in 2040.^[1]

Toxoplasma gondii is spread all over the world and causes toxoplasmosis, which is a major parasitic disease. A wide range of hosts, including humans, domestic mammals, and birds, may be infected.^[1] Tissue cysts formed during infections are controlled by the humoral immune system and cellular immunity.^[1] TH1 lymphocytes

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and macrophages are constantly stimulated by parasitic antigens, in which gamma interferon plays a key role.^[3] As a result, the resulting cysts become dormant and inactive in the tissues for a long time.^[4] *T. gondii* often presents with congenital complications (such as hydrocephalus, preterm birth, and abortion) as well as neuropathic abnormalities, which previously predominated in high-risk populations, such as pregnant women and those with immunodeficiency or those taking immunosuppressive drugs^[5] are found to have a defective cellular immune system and are at high risk for reactivation of chronic *Toxoplasma* infection, which the process may also occur in inflammatory patients.^[5] Existing data suggest a potential role for inflammation in the pathogenesis of T2DM.^[6] Determining the prevalence of anti-*Toxoplasma* antibodies in diabetics largely indicates their risk because these individuals are deficient in cellular immunity; the number of neutrophils, monocytes, and macrophages in the peripheral blood are reduced and their function is impaired. They are at high risk for many diseases and susceptible to toxoplasmosis. In other words, *T. gondii* acts as an opportunistic agent in immunocompromised individuals.^[5] The risk of activation and relapse of the disease is high in immunocompromised patients. Patients suffering from cancer, patients with organ transplant, on-dialysis patients, and patients who take immunosuppressants are at risk of toxoplasmosis.^[7]

Due to the importance of toxoplasmosis and its impact on diabetes, it has not been controlled and no study has been conducted on the prevalence of toxoplasmosis among diabetics in Zabol, southeastern Iran, with special weather and location. For this purpose, anti-*T. gondii* immunoglobulin (Ig) G and IgM antibody titers were determined in the blood serum of diabetics and healthy people as a control in the city of Zabol. Some demographic characteristics and risk factors associated with seroprevalence of toxoplasmosis in T2DM patients were also examined.

MATERIALS AND METHODS

Study area and population studied

In this cross-sectional study, patients with T2DM and healthy individuals (control) referred to the laboratories of public hospitals in Zabol city were examined.

Zabol is the capital of Zabol County, Sistan, and Baluchestan provinces in southeastern Iran. Located on the border with Afghanistan, Zabol was known as Sistan until the late 1920s. The climate of the area is 28°C temperature with 42 km/h NW wind, and 25% humidity, and Zabol is close to Lake Hamun and the Hirmand River that irrigates the region. Lake Hamun is a seasonal lake that is often dry. Zabol is shut to Lake Hamun and the Hirmand River which irrigate

the region. Lake Hamun is a seasonal lake that is frequently dry.^[1]

At first, the necessary coordination was made with reference laboratories, Imam Khomeini Hospital, and the Diabetes Laboratory of Al-Zahra Charity Center in Zabol to start the study and collect the samples.

In the sampling method, a systematic random approach method was used, in this way, blood was taken from the first person, blood was not taken from the next two people, and blood was drawn from the fourth person.

After obtaining consent, we write down the details of the people referring to the public treatment centers and laboratories of Zabol city including the control group. Inclusion and exclusion criteria were determined to like definite disease which was mandatory for the case and voluntary participation for the case group and control group, and no diseases with dietary restrictions, lack of pregnancy or breastfeeding of women participating in the study, located in the age range of 21–75 years for T2DM, no chronic complications of diabetes (retinopathy and nephropathy), no chronic complications of diabetes in the week leading up to the study, nonparticipation in any specific dietary program in the 2 months leading up to the study, no dietary supplements, and the selection of the case and control groups were determined by matching. Some limitations had been associated with emigration; we had to select another patient or control person. They were given a questionnaire to the patient and control group that included characteristics, including the name and surname, age, sex, place of residence (urban or rural), contact with the cat, consumption of undercooked meat, and take blood samples. Some were excluded from the study due to a lack of entry requirements and a total of 720 people were evaluated in this study. At first, blood samples were collected and after centrifugation, serum was separated from blood cells and in prenumbered 5cc microtubes, and diabetes and healthy subjects (control) were divided into two groups: type of diabetes and healthy subjects (control).

A serum glucose test is performed by professional staff and normal serum glucose samples are selected as a control or control sample. If people have high serum glucose in the range of 200 and above, they are considered diabetic. A fasting glucose (glucose) test after at least 8 h of fasting is considered diabetes with a result of 126 mg/dL, or a glycosylated hemoglobin test ≥ 6.5 , or a 2-h glucose test that measures blood glucose after at least 8 h of fasting. Then, 2 h after eating, the glucose solution is measured and a result higher than 200 mL/dL is considered diabetes.

According to the general symptoms of diabetes, age, history, and if the result of the test for glycosylated hemoglobin

(HbA1C) was higher than 6.5 a person was considered to have diabetes. The blood serum of the subjects with high glycosylated hemoglobin content, confirmed as diabetes, was collected from these subjects and assigned to the control group and poured into 5 mL sealed tubes and counted and stored in the freezer (-30°C) until the test was performed.

Serologic methods

Serological testing is performed by antigen/antibody enzyme-linked immunosorbent assay (ELISA) technique using a Pishtaz tab kit made in Iran, and this kit has a sensitivity of 95%. Blood serum samples were diluted with buffer solution in a ratio of 1–101 as per the kit instructions. In 5 wells in the first row, we poured the standard, and in the next 3 wells, blank, positive control serum, and negative control serum, respectively. The control serum is poured into two subsequent wells with a rate of 100 μL and other wells up to house 96 are used for samples. After covering the wells with the plate label, the wells were incubated for 30 min. In the next step, the contents of the wells were emptied and the wells were washed 5 times with a washing solution, each time washing with about 300 μL of washing solution. Pour each well and then empty the wells by turning them upside down and shaking them, and at the end of the washing step, place the wells up-side down with gentle blows on a damp paper to remove excess droplets. Then, pour 100 μL of the ready-to-use conjugated enzyme into the wells, except for the control well. After covering the wells with the plate label, the wells are incubated for 30 min, the contents of the wells are emptied and washed 5 times with ready-to-use washing solution, and then, 100 μL of dye solution were added to all wells, including blank wells. They were incubated for 15 min at room temperature and in the dark. Finally, enzymatic reactions were stopped by adding 100 μL of the stopper solution to each well. To measure the light absorption of each well, an ELIZA reader device was used. The standard light absorption of the samples were read with the help of an ELISA-R device at a wavelength of 450 nm and, if possible, against a filter frequency of 630 nm.

Statistical analysis

Results were analyzed using SPSS-19 (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp) and Chi-square test and Fisher's exact test to compare statistical parameters and seroprevalence values. If $P < 0.05$, it was considered the level of significance.

RESULTS

In this study, 720 blood samples of patients with T2DM and healthy individuals (control) referring to government laboratories in Zabol City, including the central laboratory,

Imam Khomeini Hospital (RA), and Al-Zahra Charity Center Diabetes Laboratory during the period from October to January 2017 were examined. Among 360 with T2DM, 216 (60%) were positive for IgG antibodies and 144 (40%) were IgG negative and 9 (2.5%) were IgM positive and 351 (97.5%) were IgM negative for *T. gondii* antibodies. In the control group, 173 (48.1%) were IgG positive and 187 (51.9%) were IgG negative. Furthermore, 1 (0.3%) was IgM positive and 359 (99.7%) were IgM negative.

According to the Chi-square test, a statistically significant relationship was found between the IgG result in the experimental and control groups in patients with T2DM ($P < 0.05$) [Figure 1]. This shows that patients with T2DM might be more susceptible to toxoplasmosis.

According to Fisher's exact test, a statistically significant relationship was found between IgM results in the experimental and control groups in patients with T2DM [Figure 2] ($P < 0.05$).

Regarding T2DM, according to the results of the Chi-square test in the control group, a statistically significant relationship was observed between positive and negative cases of IgG and sex ($P < 0.05$) but there is no statistical relationship in the experimental group. Furthermore, in males, the two groups' positive and negative IgG cases were recorded statistically ($P < 0.05$) but no statistical relationship was found in females [Table 1].

Furthermore, no statistical relationship was found between the two groups according to gender and positive and negative cases of IgM ($P > 0.05$) [Table 2].

There was a significant relationship between the two groups regarding contact with cats based on the Chi-square test ($P < 0.05$) [Figure 3].

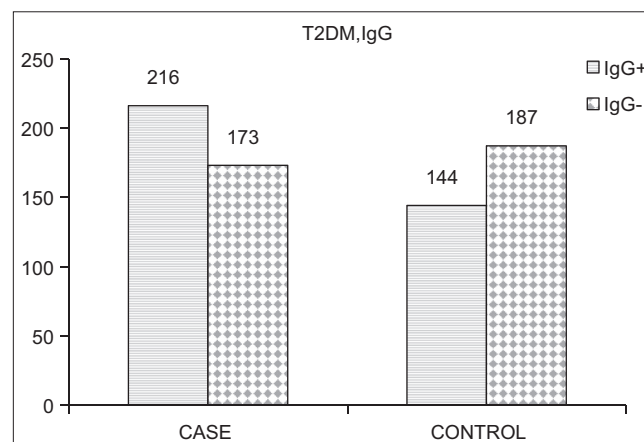


Figure 1: Number of seropositivity of the *Toxoplasma gondii* IgG antibodies in diabetic patients and healthy control ($P = 0.001$). IgG = Immunoglobulin G, T2DM = Type 2 diabetes mellitus

Based on the Chi-square test, no statistically significant relationship was found between the place of residence in both positive and negative groups ($P > 0.05$) [Figure 3].

Based on the Chi-square test, a statistically significant relationship was found between the level of education in the two groups ($P = 0.05$) [Figure 3].

DISCUSSION

In several studies, the relationship between diabetes and toxoplasmosis has been challenged, and the present study was designed to verify the presence or absence of a relationship between type 2 with toxoplasmosis. In this study, the ELISA method was used. T2DM is on the rise in both developed and developing countries. The results of this study showed that there is a connection between T2DM and toxoplasmosis infection.

The main purpose of this study was to evaluate the serum prevalence of IgG and IgM antibodies against *T. gondii* in the blood of diabetic patients referred to health centers in

Zabol city. This study was conducted for the first time in Zabol city.

In the present study, the prevalence of IgG and IgM anti-*T. gondii* antibody titers in T2DM patients and healthy individuals (control) were examined from a total of 720 samples, including 360 T2DM patients and 360 healthy individuals. Among T2DM, 216 were (60%) IgG positive while in the control group (without diabetic disease) the number of healthy IgG-positive individuals was estimated at 173 samples (48.1%). This significant relationship indicates that T2DM is a risk factor and an increased risk factor for toxoplasmosis.

A study was conducted by Jafari Madrak *et al.* on diabetic patients with a control group on 205 people (42 men and 163 women) with an average age of 60-13. Among patients, 60 (29.3%) were IgG negative and 145 (70.7%) were positively sensitive (36.6% in the acute phase (IgG+, IgM+), 49.6% in the chronic phase (IgG+, IgM-), and 13.8% (IgG-, IgM+) false positives.^[8] Similarly, Li *et al.* (2018)^[5] reported a high *Toxoplasma* seroprevalence (41.5%) in T2DM patients compared to controls (24%).

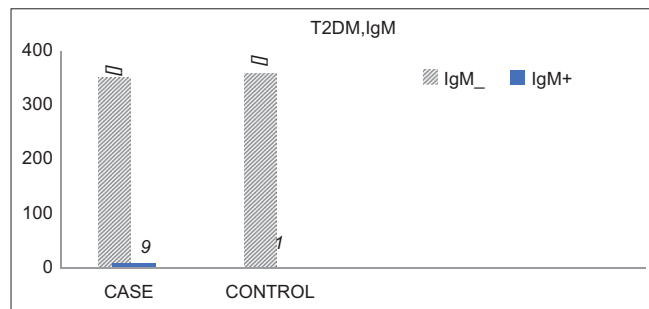


Figure 2: Number of seropositivity of the *Toxoplasma gondii* IgM antibodies in diabetic patients T2DM and nondiabetic healthy control ($P = 0.004$). IgM = Immunoglobulin M, T2DM = Type 2 diabetes mellitus

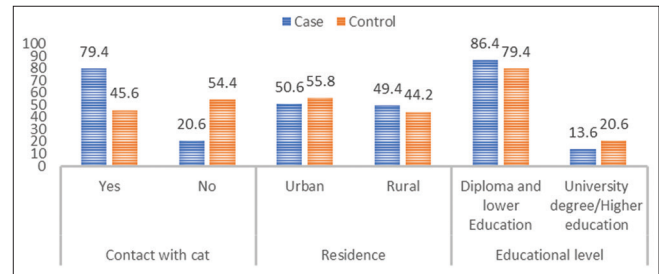


Figure 3: Demographic characteristics and risk factors related to seroprevalence of toxoplasmosis in T2DM patients. T2DM = Type 2 Diabetes Mellitus

Table 1: Chi-square test results for sex in people with type 2 diabetes and anti-toxoplasmosis immunoglobulin G antibody in case and control groups

Parameters	Males			Females			P
	NT (%)	NP (%)	Percentage	NT (%)	NP (%)	Percentage	
Total number							
Group							
Control	169 (46.9)	72 (20)	42.6	191 (53.1)	106 (29.4)	54.1	0.000
Case	135 (37.5)	80 (22.2)	59.3	225 (62.5)	136 (37.8)	60.4	0.824
P		0.004			0.308		

NT=Number tested; NP=Number positive

Table 2: Fisher's exact test results for sex in people with type 2 diabetes and anti-toxoplasmosis immunoglobulin M antibody in case and control groups

Parameters	Males			Females			P
	NT	NP	Percentage	NT	NP	Percentage	
Total number							
Group							
Control	145 (40.3)	0	0	215 (59.7)	1	0.5	1.000
Case	168 (46.7)	3 (0.83)	1.8	192 (53.3)	6 (1.7)	3.1	0.511
P		0.252			0.056		

NT=Number tested; NP=Number positive

In addition, Li *et al.* (2018) in China confirmed an appreciably greater *Toxoplasma* seroprevalence in T2DM sufferers (23.5%) than in the control team (11.75%) ($P < 0.001$).^[5]

Over the previous two decades, direct and oblique associations between irritation and T2DM have been elucidated, suggesting that this kind of DM is a continual inflammatory ailment. For example, T2DM development has been increasingly more correlated with dysfunction and/or necrosis of cells with the aid of reactive oxygen species, nitric oxide, and some pro-inflammatory cytokines, main to feasible insulin resistance in animals.^[5]

It is not clear whether reactivation of latent or dormant infection increases the Immunoglobulins level so that we can diagnose the disease or if they are more susceptible to reinfection. However, some studies hypothesize that toxoplasmosis opens the way for diabetes and that diabetes clears the way for toxoplasmosis, but this depends on which one creates, to begin with.^[9] Some articles point to an increase in toxoplasmosis infection in diabetic people, and some consider toxoplasmosis to be the cause of diabetes because it attacks the nucleated cells of the pancreas, and some articles refer to both.^[9]

Furthermore, it appears that the activation of latent toxoplasmosis can raise Ig levels, increase the proportion of seropositive individuals, or, as noted in the majority of publications, make persons with diabetes, particularly type 2, more susceptible to the condition.

In our study, there was a statistically significant relationship between positive and negative IgG and gender ($P < 0.05$) but there is no statistical relation within the experimental group [Table 1]. Furthermore, in males between the two groups, positive and negative IgG cases were recorded statistically ($P < 0.05$).

Regarding T2DM, according to the results of the Chi-square test in the control group, a statistically significant relationship between positive and negative cases of IgG and sex ($P < 0.05$) was observed, but there is no statistical relationship in the experiment group. The two groups of positive and negative IgG cases were also, significant relationship statistically recorded in men ($P < 0.05$), but no statistical association was found in women.

In one study in Mexico,^[11] stratification by gender showed similar frequencies of *T. gondii* infection among females (7/107: 6.5%) and controls (4/107: 3.7%). The frequency of *T. gondii* infection in males (3/49: 6.1%) was similar to that observed in control males (1/49: 2.0%). The contentions between distinctive things may be due to the

estimate of the tests and the occupancy of the tried members, whether from the country or civic regions, and the contact with the hazard variables.^[11]

In another study, Elkholy *et al.* showed that *Toxoplasma* IgG seropositivity was in 67/90 (74.4%) diabetics and 30/90 (33.3%) controls with a statistically significant difference ($P < 0.05$). Among diabetics, IgG seropositivity was recorded in 17/22 (77.3%) of T1DM cases and 50/68 (73.5%) of T2DM cases. The difference between the two diabetic groups were statistically significant ($P < 0.05$). A statistical significance was found between all studied groups regarding age and cat contact ($P_1 \leq 0.05$).^[10] In addition, Khattab *et al.* (2019) reported other risk factors consisting of place of residence ($P < 0.001$) and exposure to cats and soil ($P < 0.0001$).^[11] Accordingly, in the present study, we found a significant association between cat contact and the level of education in M2TD (p -value <0.05) [Figure 3].

However, some studies concluded that there was not found a significant relationship between T1DM and T2DM and toxoplasmosis.^[12] Following our study, one study that was achieved in Tehran, finding indicated that toxoplasmosis could be considered a possible risk factor for T2DM, while no statistically significant association was found between *T. gondii* infection and T1DM.^[13] On the contrary, some studies indicated that they found a significant relationship between T1DM and toxoplasmosis.^[14]

A study conducted by Shirbazou, *et al.* in 2013 in Iran used the ELISA method. The anti-*Toxoplasma* IgG antibody titer was 60.43% in diabetic patients and 38% in the control group. Thus, diabetes increases the risk of toxoplasmosis twice as much as in healthy people.^[15]

Positive IgM antibody titer in T2DM people (2.5%) and IgM negative (97.5%) compared with the control group was found to be meaningful statistics in the T2DM patients. Numerous shreds of evidence and studies have shown those various infections, including *T. gondii*, easily occur in patients with T2DM. Toxoplasmosis should continue to be considered a potential contributor to the development of T2DM disease.^[10]

The potential link between T2DM and *T. gondii* sometimes leads to *Toxoplasma* infection, which is sometimes said that this parasite increases susceptibility to T2DM. The pathological process of T2DM has been recognized as a current deterioration of the internal secretion humor capability of duct gland β cells that do not permit compensation for a redoubled peripheral insulin demand.^[11]

Toxoplasma parasites on the other hand can invade the β pancreatic cells and the presence of *T. gondii* pancreatic

infection was reported in both humans and animals. In a series of 18 autopsy cases of toxoplasmosis acquired in New York, three cases involved a disseminated *T. gondii* recovered from the pancreas. As a result, impaired insulin secretion causes diabetes and inflammation of the pancreas.^[1]

However, in a 2017 study by Nassief Beshay *et al.* in Egypt, the association of *T. gondii* as a possible pathogen of diabetes in mice, the frequency of anti-*T. gondii* IgG-positive antibodies in T1DM 86.37% and in T2DM 66.67%, and the control group reported 60%, which was statistically significant ($P < 0.005$). Therefore, patients with *T. gondii* may be at higher risk for diabetes than noninfected individuals.^[16]

A study conducted by Hamidreza Majidiani *et al.* in 2016 at Tarbiat Modares University in Tehran by systematic method and meta-analysis to investigate the possible association between chronic toxoplasmosis and diabetes using English literature databases (Google, PubMed, Web Science, Ingentaconnect, ProQuest, Ovid, Scopus, ScienceDirect, and Wiley online library) was searched for 95% odds ratio and 95% confidence interval. The incidence in people with T1DM was 1.1, while the ratio in patients with T2DM was 2.39, so the risk of developing T1DM and T2DM was due to exposure to *T. gondii* as high as 1.1 and 2.39 times related to those who were not affected by the parasite, respectively.^[17]

The present study shows a range almost identical to previous studies such as Shirbaz, ^[15] and toxoplasmosis was observed in 60.43% and 38.7% of diabetic cases and health controls, respectively.

The high prevalence of *Toxoplasma* parasites in people with T2DM due to an immunodeficiency system leads to the release of bradyzoite from tissue cysts. The proliferation of tachyzoites leads to the activation of dormant infection, so screening may be helpful in this regard, as in the case of gestation diabetic women.^[18]

CONCLUSION

Anti-*Toxoplasma* antibodies including IgG and IgM were higher in diabetic patient in comparison to control group.

Ethics ID approval

All procedures followed complied with the relevant Human Experimentation Commission institutional ethical standards and versions since 1964, Declaration of Helsinki. Informed consent was obtained from all patients included in the study. After obtaining the necessary approval number, Code of Ethics was approved with the Ethics ID IR. ZBMU. REC.1400.074 and Consent, the samples were collected and centrifuged to separate serum.

Authors' contribution

MD and MS. designed the study and designed the study protocol; MD. was the supervisor; SB. was the statistics consultant and MS. was the MSc student of this study. All authors have examined and accredited the last model of the manuscript.

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Conflicts of interest

The authors declared no conceivable conflicts of activity concerning the research, authorship, and/or e-book of this article.

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