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Prevalence of toxoplasmosis in Iraqi patients with diabetic type 2

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ABSTRACT

Background. The obligate intracellular protozoan parasite Toxoplasma gondii is a member of the phylum Apicomplexa. In people with impaired immune systems, it can result in opportunistic infections. Diabetes mellitus is regarded as a metabolic disease that raises the host's vulnerability to and risk from several infections, including T. gondii infection. The main object of this study is to ascertain the toxoplasmosis seroprevalence and correlation among Iraqi patients with type 2 diabetes.

Methods. The level of Toxoplasma antibodies IgG, IgM, and IgA was measured in 109 samples of Iraqi diabetes type 2 patients using immunochromatography rapid test, CMIA, and ELISA. Eighty samples, considered to be a healthy control group, were collected from a private laboratory in Baghdad, Iraq, between March 2022 and June 2022, with an age range of 15 to 85 years.

Results. Comparing the diabetes patient group to the non-diabetic control group, the results showed that the diabetic patient group had the highest mean glucose levels in their fasting test (174.55 ± 3.96 mg/dL), random test (216.89 ± 4.96 mg/dL), and HbA1C (7.1 ± 0.178), respectively. Furthermore, the study demonstrated that, in an immunochromatography fast test, the group of diabetic patients had the largest distribution of IgG anti-Toxoplasma when compared to the non-diabetic control group. Additionally, all samples were seronegative for anti-Toxoplasma IgM and IgA, with significant differences in CMIA and ELISA. However, 51/109 (46.79%) and 30/80 (37.5%) of the diabetes group and the non-diabetic group, respectively, showed seropositive for anti-Toxoplasma IgG.

Conclusion. This study found that no acute toxoplasmosis detected in the studied cases while chronic toxoplasmosis detected among diabetic type 2 patients.

Keywords: toxoplasmosis, diabetes mellitus (type 2), HbA1C, IgG, IgM and IgA

INTRODUCTION

Toxoplasma gondii, a protozoan parasite that is a member of the Apicomplexa phylum, is the source of the common zoonotic disease toxoplasmosis. Up to one-third of all people on the planet are thought to be infected with T. gondii. This parasite's capacity to spread quickly is mostly due to its life cycle [1]. The obligatory intracellular protozoan *T. gondii* infects the feline family. The asymptomatic form of infection is the most prevalent in people [2-4]. However, it has been common and leads to opportunistic infections in immunocompromised people, including hemodialysis patients and those with psoriasis [5,6]. Reticulated cell hyperplasia and lymphadenopathy are the typical symptoms of this infection [2]. *T. gondii* infections can result in acute or chronic cases, with or without symptoms. The disease's symptoms and complications primarily manifest in the acute infection, which is followed by an immune system activation, control of the parasite's proliferation, and the formation of tissue cysts in the host's neuromuscular tissues [3]. Additionally, since *T. gondii* can be passed from an infected mother to the fetus through the placenta (transplacental) or after vaginal birth, infection with the parasite can result

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in fetal death [7-9]. Pregnancy-related congenital toxoplasmosis can result in hydrocephalus, blindness, deafness, spontaneous abortion, stillbirth, and varying degrees of mental or physical disability. Numerous immunological and molecular techniques can be used to diagnose T. gondii infection [10,11].

Diabetes is a metabolic disease that develops when the body is unable to use insulin as it should. The pancreas secretes insulin, an anabolic hormone that helps cells absorb glucose from diet. Lack of insulin causes glucose to build up in the blood, which raises blood sugar levels and decreases the amount of energy that cells can produce. The patient experiences weariness as a result [11-13]. Approximately 90% of all diagnosed instances of diabetes are type 2 diabetes, commonly referred to as non-insulin independent diabetes mellitus. This is the most common kind of the disease. Due to chronic hyperglycemia, this illness impairs humoral and cellular immune response and may accelerate the development of latent opportunistic infections. According to predictions, the number of individuals living with diabetes is expected to approach epidemic proportions, with 643 million expected to have the condition by 2030 and 783 million by 2045 [12].

In Iraq and other countries, toxoplasmosis and type 2 diabetes are highly prevalent [14]. Despite this, little attention has been paid to *T. gondii* infection in type 2 diabetes [15]. Patients with diabetes have a weakened immune system, making it unable to block parasite replication. This results in tissue cyst formation in most bodily tissues, with a high concentration in the central nervous system, skeletal and cardiac muscles, and no discernible clinical symptoms [16].

However, a serious and perhaps lethal course of T. gondii infection can happen in immunocompromised individuals. Weakened humoral and cellular immunity, as well as conditions like cancer, immunosuppressive medications, corticosteroids, radiation, and splenectomy, which can reactivate T. gondii in a chronic state, can all contribute to this. The host's immune response to the T. gondii infection is mostly dependent on cell-mediated immunity, and the development of a cell-mediated inflammatory response is triggered by a dominant T-helper type 1 response [17].

The main object of this study is to determine the seroprevalence of toxoplasmosis in Iraqi diabetic type 2 patients and the relationship between them.

MATERIALS AND METHODS

Subjects and Samples

In this study, 109 samples of Iraqi patients with type 2 diabetes who were treated at a private laboratory in Baghdad, Iraq, that specialized in diabetic testing and were diagnosed by physicians were included. Between March and June 2022, 80 samples of non-diabetic people were compared; their ages ranged from 15 to 85 years old, with a mean (49.9±1.29). Each sample was given five milliliters of venous blood, which was then put in a gel tube, the serum of which was separated, and utilized in the analytical diagnostic tests for toxoplasmosis and diabetes.

Diabetes mellitus diagnosis

Blood glucose is assessed using a fasting test, a random test, and a hemoglobin A1C Architect kit (Abbott GmbH, Germany) that is used in accordance with the manufacturer's instructions to measure glycated hemoglobin level.

T. gondii diagnosis

First, *T. gondii* was identified using the Toxoplasma IgM/IgG antibody rapid test (Immunochromatography) kit (Qingdao Hightop Biotech Company, China) in accordance with the manufacturer's protocol. Next, the level of Toxoplasma antibodies IgG and IgM was measured using the chemiluminescent microparticles immunoassay (CMIA) Architect Toxo IgM/G kit (Abbott GmbH, Germany) and the Sandwich Enzyme-linked Immunosorbent assay (ELISA) kit (mybiosource Inc., USA) in accordance with the manufacturer's instructions.

Statistical Analysis

The statistical analysis system (SAS) program [18] was used in this investigation to examine how different circumstances affected the study's parameters. The analysis of variance (ANOVA), commonly referred to as the Least Significant Differences (LSD) test, was used to compare means and establish statistical significance. With a probability of 0.05 or 0.01 the Chi-square test was used to compare percentages and evaluate significance.

RESULTS

Diabetes mellitus diagnosis

Table 1 demonstrated that the group of diabetic patients had the highest level of glucose in all diabetic tests when compared to the healthy control group. Significant differences ($P \leq 0.05$) were observed in the fasting test, while highly significant differences ($P \leq 0.01$) were observed in the random and glycated hemoglobin tests.

T. gondii diagnosis

The *T. gondii* infection was identified by the rapid test for IgM/IgG antibodies (immunochromatog-

TABLE 1. Glucose levels of HbA1C, FBG and RBG tests in the studied cases

Groups	Total No. of samples for each group	Mean±SE of HbA1C (Glycated Hemoglobin)	Upper Value	Lower Value	Mean±SE of FBG mg/dl (FBG)	Upper Value	Lower Value	Mean±SE of RBG mg/dl (RBG)	Upper Value	Lower Value
Diabetic patients	109	7.9±0.178	15.5	5.3	174.55±3.96	300	120	216.89±4.96	410	125
Non-Diabetic Control	80	4.98±0.044	5.4	4.3	96.65±0.749	98	81	160.25±2.69	195	109
LSD value		1.667*			26.381**			31.093**		
P-value		0.0252			0.0063			0.0058		
Significant * (P≤0.05), Highly significant ** (P≤0.01).										
Reference range of HbA1C: Diabetes ≥6.5 **Reference range of FBG:** Diabetes ≥126 **Reference range of RBG:** over 200 mg/dl after two hours refers to diabetes										

TABLE 2. Detection of anti-Toxoplasma IgM/IgG antibodies in the studied groups according to rapid test (Immunochromatography test)

Groups	Total No. of samples for each group	IgG	IgM	IgG and IgM	Negative	P-value
Diabetic patients	109	45 (41.29%)	2 (1.84%)	10 (9.17%)	52 (47.7%)	0.0001**
Non-Diabetic Control	80	31 (38.75%)	1 (1.25%)	2 (2.5%)	46 (57.5%)	0.0001**
P-value		0.108NS	0.563NS	0.021**	0.544NS	–
Highly significant ** (P≤0.01), NS: Non-Significant.						

TABLE 2. Detection of anti-Toxoplasma IgM/IgG antibodies in the studied groups according to rapid test (Immunochromatography test)

Groups	Total No. of samples for each group	Toxo IgG	Toxo IgM	Toxo IgA	P-value
Diabetic patients	109	51 (46.79%)	0 (0%)	0 (0%)	0.0001**
Non-Diabetic Control	80	30 (37.5%)	0 (0%)	0 (0%)	0.0001**
P-value		0.0196*	NS	NS	–
Significant * (P≤0.05), Highly significant ** (P≤0.01), NS: Non-Significant					

raphy test), as shown in Table 2, which showed that 31/80 (38.75%) of non-diabetic controls and 45/109 (41.29%) of diabetic type 2 patients were seropositive for IgG Toxoplasma antibody with highly significant differences (P ≤0.01). Chemiluminescent microparticle immunoassay (CMIA) results indicate that 51/109 (46.79%) of diabetic patients and 30/80 (37.5%) of non-diabetic control have seropositive response for Toxoplasma IgG antibody, with significant differences. While there were no appreciable variations in the seronegative responses for IgM and IgA Toxoplasma antibodies according to the CMIA and ELISA assays, all samples from diabetic patients and non-diabetic controls were. Referring to the results of CMIA and ELISA that mentioned previously, the study groups divided into four groups according to the measuring levels of Toxoplasma antibodies as the following: diabetic patients infected with toxoplasmosis, diabetic patients only, non-diabetic individuals infected with toxoplasmosis considered as a positive control and healthy individuals considered as a negative control.

According to the chemiluminescent microparticle immunoassay (CMIA) results in Table 4, the group of diabetic patients with toxoplasmosis has the highest level of IgG antibody (34.95±7.5) UI/mL with highly significant differences when compared to other studied groups in the same assay. On the other hand, the sandwich enzyme-linked immunosorbent test (ELISA) revealed a negative reaction in anti-Toxoplasma IgM and IgA.

Age characteristics

The Table 5 clarifies that the study groups were between the ages of 15 and 85. It shows that approximately 57/109 diabetic patients with toxo-

TABLE 4. Mean titers of Anti-Toxoplasma IgG, IgM and IgA antibodies in diabetic and non-diabetic groups based on CMIA and ELISA tests

Groups	Total No. of samples for each group	Mean ± SE of Toxo IgG UI/mL	Upper Value	Lower Value	Mean ± SE of Toxo IgM UI/ mL	Upper Value	Lower Value	Mean ± SE of Toxo IgA UI/mL	Upper Value	Lower Value
Diabetic patients with toxoplasmosis	51	34.95±7.5 a	217	0.6	0.082±0.0052 a	0.2	0.02	0.154±0.029 b	0.82	0.02
Diabetic patients	58	0.024±0.058 b	2.3	0.0	0.072±0.003 a	0.16	0.02	0.29±0.032 ab	0.62	0.04
Toxoplasmosis asymptomatic individuals (control positive)	30	32.7±8.45 a	230	5.8	0.10±0.04 b	0.19	0.01	0.49±0.06 a	0.34	0.07
Healthy individuals (control negative)	50	0.38±0.055 b	2.5	0.0	0.042±0.005 ab	0.13	0.01	0.47±0.063 a	0.15	0.06
LSD value		0.218*			0.0595*			4.227**		
P-value		0.0392			0.0478			0.0001		
Means having with the different letters in same column differs significantly Significant * (P≤0.05), Highly significant ** (P≤0.01)										
Reference range of Toxo IgM: Primary (acute) infection≥0.6 **Reference range of Toxo IgG:** Secondary (chronic) infection≥3.0 **Reference range of Toxo IgA:** Positive indexes if higher than 1.1										

TABLE 5. Prevalence of the studied groups according to the age characteristic

Groups	Total No. of samples for each group	Male No. (%)	Female No. (%)	P-value
Diabetic patients with toxo-plasmosis	51	25 (49.02%)	26 (50.98%)	0.889NS
Diabetic patients	58	31 (53.45%)	27 (46.55%)	0.599NS
Toxoplasmosis asymptomatic individuals (control positive	30	17 (56.66%)	13 (43.34%)	0.465NS
Healthy individuals (control negative)	50	32 (64.00%)	18 (36.00%)	0.0477*
P-value		0.142NS	0.322NS	–
Significant * (P≤0.05), NS: Non-Significant.				

TABLE 6. Distribution of the studied cases according to the sex characteristic

Age range (Year)	Diabetic patients with toxoplasmosis		Diabetic patients		Toxoplasmosis asymptomatic individuals (control positive		Healthy individuals (control negative)		P-value
	No	%	No	%	No	%	No	%	
15-30	0	0.0	2	3.45	4	13.33	16	32.00	
31-45	5	9.81	11	18.96	11	36.67	15	30.00	
46-60	16	31.37	18	31.04	8	26.67	10	20.00	0.0001**
61 ≤ 85	30	58.82	27	46.55	7	23.33	9	18.00	
Total	51	100	58	100	30	100	50	100	
Mean±SE	62.78±1.554		56.88±1.76		43.1±2.90		40.76±1.83		
Highly Significant ** (P≤0.01)									–

plasmosis (30/51; 58.82%) or without toxoplasmosis 27/58 (46.55%) groups belong to the age range of 61-85, while approximately 11/30 (36.67%) of the positive control group that has toxoplasmosis is within the age range of 31-45. Additionally, 1 6/50 (32.0%) of the healthy negative control group were in the 15-30 age range. Additionally, the group of diabetic patients with toxoplasmosis infection had the greatest age mean with very significant differences.

Sex characteristics

Table 6 demonstrates that approximately 26/51 (50.98%) of diabetes patients with toxoplasmosis are female. Conversely, male sex accounted for 31/51 (53.45%) of diabetic individuals who did not have toxoplasmosis. Nonetheless, male gender comprised 49/80 of the positive and negative control groups, with no statistically significant differences.

DISCUSSION

A plausible correlation between toxoplasmosis and diabetes may have clinical implications, providing insight into the intricate pathophysiology of the disease. The current theory generally holds that toxoplasmosis increases the risk of contracting diabetes or, conversely, that toxoplasmosis infections are more common in diabetic people [19].

The fasting blood glucose results are similar to those of Al-Aubaidi et al. [20], who showed that there are very significant disparities in the fasting test glucose levels between the diabetes patient groups (305.4±13.5) and the case control group (111.4±2.1). The findings of this investigation, however, are consistent with those of Elkholy et al. [21], who showed that only 15% of toxoplasmosis patients had glycated hemoglobin in their HbA1C test results. A commonly used measure for long-term glycemic control is the glycosated hemoglobin test [22]. Currently, one of the best methods to assess if diabetes is under control is to use the HbAlc test [23].

The results of the current immunochromatography test, however, are similar to those reported by Al-Khafajii [24], who demonstrated that whereas 22/45 (48.88%) of the diabetic patients were seropositive for anti-Toxoplasma IgG antibody, 28/55 (50.9%) of the non-diabetic control group were seropositive for the same antibody. The results of this study also agreed with those of Al-Aubaidi et al. [20], who reported that 47 out of 100 diabetic patients had a positive Toxoplasma IgG antibody test result, with highly significant differences between them and a healthy control group that tested negative for the same antibody. The immunochromatography test has been contemplated as a potential replacement screening technique for toxoplasmosis detection due to its lower cost compared to alternative tests, ease of use (results can be obtained in 15 minutes), and lack of additional equipment or training requirements [25].

The results for Toxoplasma IgG, IgM, and IgA are comparable to those of Ozcelik et al. [26], who found that 108/200 (or 54.0%) of patients with type 2 diabetes were seropositive for Toxoplasma IgG antibodies. Nevertheless, a different study discovered that 24 people out of 200 (14.0%) were seropositive for Toxoplasma IgM antibodies. Additionally, Younis and Elamami [27] findings showed that every one of the 200 diabetic patient samples tested positive for Toxoplasma IgG, IgM, and IgA antibodies in 38/200 (41.5%), 21/200 (10.5%), and 7/200 (3.5%) cases, respectively. These findings are consistent with the findings of this investigation about anti-Toxoplasma IgG. Whereas, disagree with the anti-Toxoplasma IgM and IgA results.

The findings of this investigation are in line with those of Hamida et al. [28], who reported that all samples of diabetes patients and controls were seronegative for IgM Toxoplasma antibody, and that 14/37 (or 37.8%) of diabetic type 2 patients had positive IgG antibodies. Furthermore, the current study's findings are consistent with those of Goekce et al. [29], Alvarado-Esquivel et al. [30], and Molan & Ismail [31], who identified *T. gondii* infection in people with type 2 diabetes.

IgG antibodies are linked to chronic infections, while IgA and IgM antibodies are associated with recent infections (acute infections). Toxoplasmosis infection has the power to change the course of chronic diseases. However, the IgA antibody test is primarily utilized to identify congenital infections and is not commonly performed. As a result, the IgA antibody test is a crucial diagnostic for identifying T. gondii infection in infants [32-34].

Additionally, the results of Table 5 in this study are consistent with those of Khalil et al. [35], who found that roughly 37/90 of diabetes patients with age range of 50-65. This indicates that age is a significant factor in both diabetes mellitus and toxoplasmosis infections because older adults have accumulated exposure to various *T. gondii* risk factors. Moreover, diabetes can lead to toxoplasmosis as well as the other way around if it compromises the immune system [28,36].

Furthermore, results of table 6 concur with those of Kuba et al. [37], which found that 116/150 (77.33%) of diabetic patients without toxoplasmosis are female. In contrast, results of table 6 conflict with those of the same study [37], which found that 81/97 (83.51%) of diabetic patients with toxoplasmosis are female.

The latest research suggests latent toxoplasmosis in diabetes people. As a result, bradyzoites can be found throughout the host's life in a variety of bodily regions, including the central nervous system. It has also been noted that certain microbes become more virulent in diabetes cells [26,28]. However, there's a chance that diabetes and toxoplasmosis will interact. Given that the pancreas is important in insulin secretion and that its suppression can cause the formation of diabetes, the appearance of necrotic lesions in the pancreas of experimental animals infected with *T. gondii* raises the possibility that tox-

oplasmosis may play a role in the development of diabetes. Prior research has demonstrated that *T. gondii* infection in lab animals can cause inflammation and necrotic lesions in a number of organs such as the stomach, pancreas, lymph nodes, and intestine, and tachyzoites have been identified in these lesions [17,36,38].

Because of this, diabetic individuals who already have impaired immune systems may experience serious neurological issues due to the reactivation of latent infections like epilepsy, schizophrenia, and traffic accidents. Furthermore, due to their weakened immune systems, individuals are more susceptible to eye conditions such cataracts and chorioretinitis [39]. According to Oz [40] study, *T. gondii* nucleated cells, which include those in the pancreas, can kill β cells, which reduces insulin release and raises the risk of diabetes, acute and chronic pancreatitis, and other related conditions.

Moreover, pancreatic tissue necrosis has been linked to acute toxoplasmosis [40]. Bradyzoites of *T. gondii* have been found in acinar cells, bile duct epithelial cells, and tissue cysts in pancreatic tissue [41].

Furthermore, T2DM has been described as an inflammatory chronic illness that alters immune cell activity in a number of ways [38]. This study's data indicates that latent *T. gondii* is comparatively common in Iraqi diabetic patients. Since diabetes weakens the immune system, latent *T. gondii* can lead to a number of issues.

CONCLUSION

This study found that no acute toxoplasmosis detected in the studied cases while chronic toxoplasmosis detected among diabetic type 2 patients.

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Ethical clearance:
Ethics of scientific research were carried out in accordance with the international conditions followed in dealing with laboratory animals, and included animal health, husbandry and care for it, and providing appropriate conditions for it in terms of food, and appropriate methods were adopted in dealing with it when experimenting, and this is consistent with the instructions of the Iraqi Ministry of Health and Environment.

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