ORIGINAL ARTICLE

Behind the Veil: Understanding Toxoplasmosis in Type 1 Diabetic Children of Menoufia Governorate, Egypt

[1]Dalia A. Shafey*, [2]Marwa F. Yousef, [3]Mona F. Salama, [4]Mai A. Elbadawi, [1]Hebatallah M. Sallam

[1]Department of Clinical and Molecular Parasitology, National Liver Institute, MenoufiaUniversity, Menoufia, Egypt
[2]Department of Epidemiology and Preventive Medicine, National Liver Institute, Menoufia University, Menoufia, Egypt
[3]Department of Clinical Pathology, Faculty of Medicine, Menoufia University, Menoufia, Egypt
[4]Department of Pediatric Medicine, Faculty of Medicine, Menoufia University, Menoufia, Egypt

Key words:
Children, Toxoplasmosis, T1DM, HBA1C, ELISA

Corresponding Author:
Corresponding author:
Dalia Shafey
Tel.: 01066946478
Dalia_shafey@yahoo.com

ABSTRACT

Background: *No doubt, type 1 diabetes mellitus (T1DM) is one of the most common endocrine and metabolic conditions in children increasing their vulnerability and risk for various infectious diseases. Toxoplasma gondii is an intracellular opportunistic protozoan parasite causing risky complications, especially in immune-compromised humans.* ***Objectives:*** *This study aimed to evaluate the seroprevalence of T. gondii (IgG & IgM) among known diabetes mellitus children with type I, and risk factors between both groups (diabetic and non-diabetic).* ***Methodology:*** *A total of 200 blood samples were taken from children who attended the Outpatient Clinic of Pediatric Endocrinology, at Menoufia University Hospitals. Blood samples were taken from 100 known T1DM patients, and 100 apparently healthy ones. All samples were examined for random blood glucose, glycated hemoglobin (HBA1C), and anti-T. gondii antibodies (IgG & IgM) by ELISA.* ***Results:*** *The results didn't show a significant difference in anti-T. gondii IgG between diabetic patients (10%) and controls (15%), without IgM positivity in either case. But seropositive IgG was significantly associated with higher HBA1C levels in diabetic patients (P< 0.001). Environmental factors such as residence, soil, and/or cat contact were major determinants of seropositivity in both groups.* ***Conclusions:*** *We conclude that T. gondii IgG seroprevalence does not significantly differ between diabetic and non-diabetic children; however, it is associated with poor glycemic control in diabetics. Environmental factors, including soil and cat contact, play a critical role in exposure risk.*

INTRODUCTION

Toxoplasmosis, a parasitic infection caused by *T. gondii* is still a significant global health issue, particularly in immunocompromised individuals, such as those with diabetes mellitus. This protozoan infection is commonly acquired through contaminated food or water or via vertical transmission from mother to fetus during pregnancy[1]. While toxoplasmosis can present asymptomatically in immunocompetent individuals, it can cause severe health complications in patients with weakened immune systems, including those with diabetes, making them particularly vulnerable to the adverse effects of this parasitic infection[2].

Diabetes mellitus, especially T1DM, exacerbates the risk and severity of infections by altering immunity[3]. Diabetic patients exhibit a reduced ability to effective immunity against *T. gondii*, increasing their susceptibility to infection[4]. Diabetic children are particularly at risk due to their age-related immune immaturity and the long-term immunological impacts of hyperglycemia, which contribute to more susceptibility to opportunistic infectious diseases, particularly toxoplasmosis, this is apart from the congenital toxoplasmosis encountered worldwide as well as Egypt[5].

In diabetic patients, hyperglycemia negatively affects neutrophil function, phagocytosis, and cytokines production for controlling parasites such as toxoplasmosis[6]. This impaired immune response can lead to severe manifestations of toxoplasmosis, such as ocular toxoplasmosis, encephalitis, or systemic infection[7]. Also, diabetes-induced complications such as nephropathy, retinopathy, and cardiovascular disease may complicate the management of toxoplasmosis in children[8].

Diagnosis and management of toxoplasmosis in diabetic children pose unique challenges. Immunological changes in diabetic patients may cause delays in the recognition of *T. gondii* infection, leading to a more aggressive disease course[9]. Early diagnosis and prompt management are essential to prevent long-

term complications in diabetic children affected by toxoplasmosis [10]. This study aimed to evaluate the seroprevalence of *T. gondii* (IgG & IgM) among known diabetes mellitus children with type I, and risk factors between both groups.

METHODOLOGY

Patient selection: This cross-sectional study was conducted on 200 children recruited from the Pediatric Endocrinology Outpatient Clinic, at Menoufia University Hospitals. Children were divided into two groups of 100 each. GI: Diabetic children (T1DM), and GII: non-diabetic healthy controls.

Ethical consideration:

The study protocol was approved by the Ethics Committee of the National Liver Institute (IRB No: 00652/2024). After explaining the aim of the study and assuring that the outcome data will be confidential, each parent gave an assigned written informed consent.

All children with T1DM under 18 years old diagnosed based on WHO criteria for diabetes classification and diagnosis (fasting plasma glucose [FPG] ≥126 mg/dL or 2 h post 75 g glucose intake [2-h PG] ≥200 mg/dL during oral glucose tolerance test or HbA1C ≥6.5% or random plasma glucose ≥200 in a patient with classic symptoms of hyperglycemia or taking any glucose-lowering medications)[11] were enrolled in the study. Those who were with T2DM, and those with diabetes secondary to post-surgical pancreatectomy, steroid therapy, cystic fibrosis, or any chronic or autoimmune diseases were excluded.

Patients in the current study were interviewed using an organized questionnaire that included the following items: name, age, gender, residence (urban/rural), and risk factors for toxoplasmosis: contact with cats and contact with soil (gardening or agricultural).

***Sample collection*:**

6 ml of venous blood was collected after fasting 8hrs under complete aseptic conditions and divided as follows: 4 ml of blood were delivered to a vacutainer plain tube, left for 30 minutes to clot, and then centrifuged for 10 minutes at 3000 rpm. Sera were separated into two aliquots, one for measurement of *T. gondii* IgG and IgM, and stored in the freezer until the test was performed. The other aliquot for measurement of random blood glucose (RBG), 2 ml was collected into an EDTA-containing tube for glycated hemoglobin (HBA1C) to confirm the diagnosis of diabetes.

The following laboratory investigations were performed: glycated hemoglobin by Roche Diagnostics (Colchester, Switzerland), RBG test was done by the autoanalyzer AU 680 (Beckman Coulter, Fullerton, USA).

Serum levels of *T. gondii* IgG and IgM were assayed using an ELISA kit supplied by Sunred Co (Sunred Co., Ltd, Shanghai, China) (Cat. No. 201-12-1845), based on the principle that certain antibodies can recognize the presence and level of antigen binding by binding to a specific target antigen. Plates were coated with high-affinity antibodies to improve sensitivity and accuracy.

Statistical analysis:

Data were collected, tabulated, and statistically analysed using an IBM-compatible personal computer running SPSS Statistical Package Version 26. Student's t-test and Chi-squared test (χ^2) were used. P-value <0.05 was considered a significant difference.

RESULTS

The anti-*T. gondii* IgG antibodies in diabetic patients and negative control were 10% & 15% respectively, but without significant differences (P> 0.05). None of the diabetic children or non-diabetic control showed seropositivity for *T. gondii* IgM antibodies. Fig 1.

A significant difference (P <0.001) was between *T. gondii* IgG and HBA1C levels but, non-significant was between HBA1C level and IgM (P = 0.163). Fig 1,2.

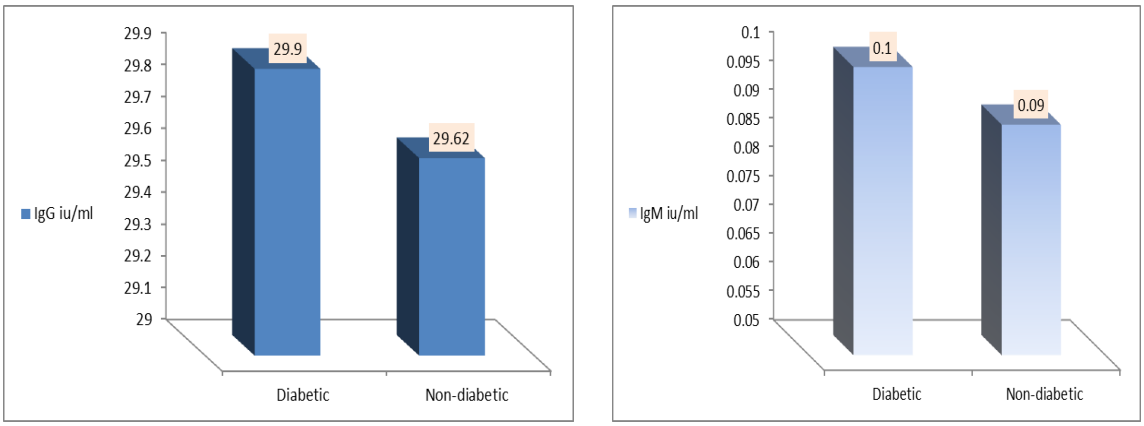
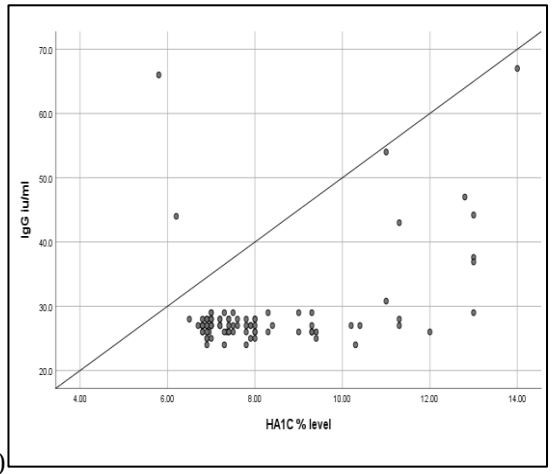


Fig. 1: IgG and IgM levels among groups

Chi-square tested qualitative values and an independent sample t-test for quantitative values, P < 0.05* significance

(a) 

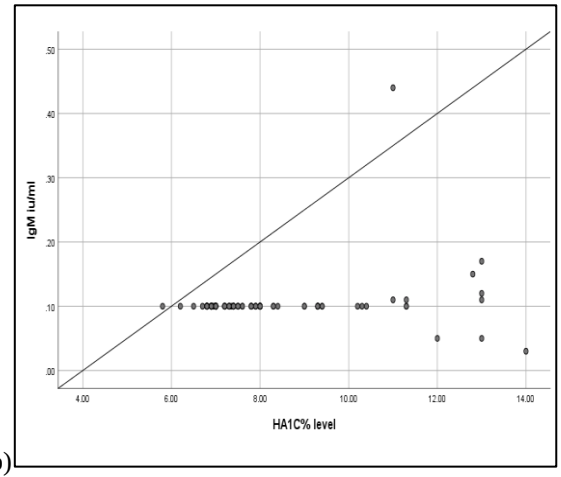
(b) 

Fig. 2: (a) Correlation between HBA1C and IgG in the diabetic group. (b) Correlation between HBA1C and IgM in the diabetic group.

Socio-demographic characters showed anti-*T. gondii* IgG positivity among T1DM children. There was a significant difference between IgG positivity & negativity as to residence, DM family history, and contact with soil and/or cats (P = <0.001, <0.001, 0.019 & <0.001 respectively), but without a significant difference as to age and sex Table 1. The negative control showed a significant difference between IgG-negative and IgG-positive as to sex, residence, and contact with soil and/or cats (P <0.001), but without significant differences as to ages. Table 1,2

Table 1: Socio-demographic factors & IgG level in the diabetic group

Parameters	IgG -ve (n=90)	IgG +ve (n=10)	Chi-square test	P value
Age in years (x ± SD)	7.77± 3.11	9.40± 4.06	1.514#	0.133
Female Male	49(56.7%) 41(43.3%)	6(60.0%) 4(40.0%)	0.112	0.738
Residence: Rural Urban	 23(25.6%) 67(74.4%)	 10(100.0%) 0(0.00%)	 22.559	 <0.001*
DM family history: Negative Positive	 44(48.9%) 46(51.1%)	 1(10.0%) 9(90.0%)	 5.499	 0.019*
Contact with soil or cats: Negative Positive	 89(98.9%) 1(1.1%)	 0(0.00%) 10(100.0%)	89.889	<0.001*

*P < 0.05 significance

Table 2: Socio-demographic factors and IgG level in the control group

Parameters	IgG -ve (n=85)	IgG +ve (n=15)	Chi-square test	P value
Age in years (x ± SD)	8.49± 2.47	9.53± 2.34	1.509#	0.135
Female Male	35(41.2%) 50(58.8%)	13(86.7%) 2(13.3%)	10.571	<0.001*
Residence: Rural Urban	 0(0.0%) 85(100.0%)	 15(100.0%) 0(0.00%)	 100.00	 <0.001*
DM family history: Negative Positive	 85(48.9%) 0(51.1%)	 15(10.0%) 0(90.0%)	 ------	 -------
Contact with soil or cats: Negative Positive	 85(100.0%) 0(0.0%)	 0(0.00%) 15(100.0%)	 100.00	 <0.001*

DISCUSSION

Toxoplasma gondii infections are among the most frequent parasitic diseases in humans. Diabetes is a hazardous disease that increases the risk of developing other diseases [3]. In terms of the qualitative results (seropositive or seronegative) for anti-*T. gondii* IgG and IgM antibodies among the study groups, the seropositivity for anti-*T. gondii* IgG antibodies in the study groups were 10% in diabetic patients and 15% in the control, but without significant difference (P>0.05).

In the present study, none of the diabetics or healthy controls tested positive for *T. gondii* IgM antibodies. Similarly, our results align with the lack of association between the incidence of *T. gondii* infection and T1DM as found in a study done by Siyadatpanah et al.[12], who reported that there was no statistically significant difference in the incidence of toxoplasmosis in diabetic and non-diabetic individuals. Also, Krause et al.[13] agreed with our study and reported significantly low levels of antibodies against *T. gondii* (P=0.001) in T1DM patients.

However, Gokce et al. [14] reported that the incidence of toxoplasmosis in diabetic patients showed a significant relation with *T. gondii* infection, where the overall seroprevalence of anti- *T. gondii* IgG was 56.62% in DM patients and 22.4% in controls. Moreover, a systematic review by Ashley et al. [15] suggests that *T. gondii* infection might be positively associated with T1DM.

According to Maryam et al.[16], there is no statistically significant difference in positive serology for anti-*T. gondii* immunoglobulins between type I and II diabetic and non-diabetic populations. On the other hand, Shirbazou et al. [17] discovered that diabetes mellitus is a significant risk factor for opportunistic infections. *T. gondii* may also be responsible for diabetes. The presence of *T. gondii* in the pancreas has the potential to directly weaken beta cells. Insulin secretion may be impaired if β cells are damaged. Contrary to our findings, Hemida et al.[18] reported that *Toxoplasma* antibodies were significantly higher in diabetic than non-diabetic Egyptian patients. Also, Khattab et al. [19] reported that anti-*Toxoplasma* IgG antibodies were 45% in type I diabetes mellitus patients and 23.3% in the controls, with a significant difference between both. Ahmed et al.[20] reported that the total seroprevalence of *T. gondii* antibodies in Minia City was significantly higher in diabetic patients (p<0.0001) than in healthy controls.

Also, in contrast to age- and gender-matched case-control research in Iran[17] and a study conducted by Elkholy et al. [21] reported a considerably high prevalence of *T. gondii* infection in diabetic patients compared to healthy individuals.

In the present study, there was a significant positive association between HBA1C level and IgG, that means patients with high levels of HBA1C > 7%, poorly controlled diabetes according to American Diabetes Association[22], are more liable for diabetes complications such as toxoplasmosis due to the occurrence of hyperglycemic-associated immune dysfunction that agreed with the results of Seino et al.[23] and Zeng et al. [3] who detected the correlation between hyperglycemia and infection.

In the present study, positive *T. gondii* IgG among diabetic patients was 40% in males and 60% in females, but without significant difference. However, in the non-diabetic males and females were 13.3% and 86.7% respectively with significant differences. This agreed with Harker et al.[24], who found that *T. gondii* seropositive rates of men and women were 6.3 and 7.2%, respectively, but without significant differences for anti- *T. gondii* IgG positivity among the diabetic patients (P=0.20, P=0.16). Also, this agreed with Soltani et al. [25], who found that the seropositivity for anti- *T. gondii* antibodies was higher in women but disagreed with Li et al.[2] found that toxoplasmosis was higher in men.

In the present study, as to contact with soil and/or cats, there was a significant difference in seroprevalence between rural and urban diabetic and non-diabetic ones. This agreed with Saadatina and Golkar [26], who found that those living in rural regions suffered from toxoplasmosis more than those living in cities. Also, there was a significant correlation between *T. gondii* infection and cat contact. This agreed with Pappas et al.[5] in the United States who reported a highly significant correlation between contact with cats and *T. gondii* infection. This disagreed with Hampton[27], who didn't find a significant correlation between those with or without cat contact.

In the present study, all diabetic patients in rural areas showed anti-*T. gondii* IgG compared to zero in urban areas (p<0.001). This agreed with Khattab et al. [19], who reported that in the rural lifestyle, there was inadequate sanitation with exposure to domestic and farm animals caused high toxoplasmosis prevalence. Yildirim et al.[28] reported that sporulated oocysts dropped from cats can contaminate the soil for several months or even the whole year.

CONCLUSION

The lack of a significant difference in IgG prevalence between diabetic and control groups suggests that *T. gondii* exposure risk may not be directly influenced by diabetes status. However, the significant correlation between IgG positivity and HbA1C levels in diabetics could imply that chronic infection impacts metabolic control or that poorly controlled diabetes

increases susceptibility to latent infections. Environmental factors (e.g., contact with soil and cats, residency) are critical in determining *T. gondii* exposure risk, irrespective of diabetes status. We might consider further exploring potential immunological mechanisms linking *T. gondii* seropositivity and glycaemic control, as well as performing multivariate analyses to control for confounding factors.

Declarations:

Consent for publication:

Not applicable

Availability of data and material:

Data are available upon request.

Competing interests:

The author(s) declare no potential conflicts of interest concerning this article's research, authorship and/or publication. This manuscript has not been previously published and is not being considered for publication in another journal.

Funding: The authors did not receive any grants from funding agencies.

REFERENCES

1. Asgari Q, Motazedian MH, Khazanchin A, Mehrabani D, Naderi Shahabadi S. High Prevalence of *Toxoplasma gondii* Infection in Type I Diabetic Patients. J Parasitol Res. 2021; 8881908**.**
2. Li YX, Xin H, Zhang XY, et al. *Toxoplasma gondii* infection in diabetes mellitus patients in China: Seroprevalence, risk factors, and case-control studies. Biomed Res Int. 2018; 1-7.
3. Zeng B, Li Y, Li Y, Guo W, Zhang Y. Immune system dysfunction in diabetes: Role of hyperglycemia and infection. Front Immunol. 2019; 10:1589-94.
4. Zhao XY, Ewald SE. The molecular biology and immune control of chronic Toxoplasma gondii infection. J Clin Invest. 2020;130(7):3370-3380.
5. Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: Global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int J Parasitol. 2017;39(12):1385-94.
6. Smith JR, Tanner AR, Hutton GL, Jones JL. Ocular toxoplasmosis and diabetes mellitus: An epidemiological analysis. Ophthalmology. 2018;125(7):1174-9.
7. Montoya JG, Remington JS. Management of toxoplasmosis. Clin Infect Dis. 2020;30(4):141-59.
8. Majumdar A, Singh S, Mukherjee R. Diabetes and its impact on toxoplasmosis: Immunological and clinical consequences. J Clin Microbiol. 2021;59(2):e2220-30.
9. Jones JL, Parise ME, Fiore AE, Ajzenberg D. *Toxoplasma gondii* infection in the United States: Seroprevalence and implications for pregnant women. Clin Infect Dis. 2018;61(5):1-10.
10. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii* from animals to humans. Int J Parasitol. 2019;30(12-13):1217-58.
11. World Health Organization (WHO). Use of glycated hemoglobin (HbA1c) in the diagnosis of diabetes mellitus. Wld Hlth Organ. 2011.
12. Siyadatpanah A, Tabatabaie F, Oormazdi H, et al. Comparison of anti-*Toxoplasma* IgG and IgM antibodies determined by ELISA method in diabetic and non-diabetic individuals in west Mazandaran Province, Iran. Iran J Parasitol. 2012;7(4):24-31.
13. Krause I, Anaya JM, Fraser A, et al. Anti-infectious antibodies and autoimmune-associated autoantibodies in patients with type I diabetes mellitus and their close family members. Ann N Y Acad Sci. 2009; 1173:633-9.
14. Goekce C, Yazar S, Bayram F, Gündoğan K. *Toxoplasma gondii* antibodies in type 1 diabetes mellitus. Turki. Klinik. J Med Sci 2008; 28, 5:619-22.
15. Ashley C, Catchpole A, Zabriskie BN, Bassett P, et al. Association between *Toxoplasma gondii* Infection and Type-1 Diabetes Mellitus: A Systematic Review and Meta-Analysis. Int J Environ Res Public Health. 2023;20(5):4436.
16. Maryam K, Mahmoud MO, Abass S, Abdolrasol S. The correlation between serum levels of anti-*Toxoplasma gondii* antibodies and the risk of diabetes. Iran J Parasitol. 2018;13(4):637-42.
17. Shirbazou S, Delpisheh A, Mokhetari R, Tavakoli G. Serologic detection of anti-*Toxoplasma gondii* infection in diabetic patients. Iran Red Crescent Med J. 2013;15(8):701-5.
18. Hemida MH, Shahat SA, Bayoumy AM, Mohamed KA, Hassan SM. Toxoplasmosis prevalence in Egyptian diabetic patients. Al-Azhar Assiut Med J. 2018;16(2):113-6.
19. Khattab HM, El Bassiouni SO, Abuelela MH, Abd Elsalam DO. Seroprevalence of *Toxoplasma gondii* among a group of Egyptian patients with type I diabetes mellitus. Bull Natl Res Cent. 2019; 43:1-7.
20. Ahmed AK, Kamal AM, AbdelFatah MM, Abd Rabou RA, Mahmoud HA, Sanadeki MM. Seroepidemiology of *Toxoplasma gondii* infection in diabetic patients and the impact of toxoplasmosis on diabetes associated complications in Minia city, Egypt. Infect Dis Trop Med 2023; 9: e1198.
21. Elkholy A, Omar R, Elbadawy A, Elawady M, Abou-Ouf E. Investigating the potential link between seroprevalence of Toxoplasma IgG and

both types of diabetes mellitus in Benha city, Egypt. PUJ. 2022;15(2):195-201.

22. American Diabetes Association Glycemic goals and hypoglycemia management: Standards of care in diabetes. Diabetes Care 2024; 47, 1: S111-7.
23. Seino Y, Nanjo K, Tajima N. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. J Diabet Investig. 2010; 1:212-28.
24. Harker KS, Ueno N, Lodoen MB. *Toxoplasma gondii* dissemination: A parasite's journey through the infected host. Parasit Immunol. 2015;37(3):141-9.
25. Soltani S, Tavakoli S, Sabaghan M, et al. The probable association between chronic *Toxoplasma gondii* infection and type 1 & type 2 diabetes mellitus: A case-control study. Interdiscip Perspect Infect Dis. 2021; 24:1-6.
26. Saadatnia G, Golkar M. A review on human toxoplasmosis. Scand J Infect Dis. 2012;44(11):805-14.
27. Hampton MM. Congenital toxoplasmosis: a review. Neonat Netw. 2015;34(5):274-8.
28. Yildirim D, Buyukboyaci NH, Bolukbasi S, et al. Investigation of *Toxoplasma gondii* seropositivity in toxoplasmosis suspected patients by chemiluminescent microparticle immunological test (CMIA). Cumhuriyet Med J. 2013;35(4):468-74.