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Association of Anti- Toxoplasma gondii seropositivity with type 2 diabetes mellitus patients in Kurdistan region, Sulaimania province, Iraq

Khoshi Tariq Sdeeq [1], Latif Omer Mohammed [2*]

[1,2] Department of Basic Medical Science, College of Medicine, University of Sulaimani, Sulaimani 46001, IRAQ

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ABSTRACT:

Toxoplasma gondii (*T.gondii*) is a zoonotic parasite that is found all over the world and can cause serious problems in people who don't have strong immune systems. People with type 2 diabetes mellitus (T2DM) often have a weak immune system and continuous inflammation, which can make them more likely to get toxoplasmosis. The point of this cross-sectional study was to find out how common *T. gondii* infection is and what factors make people more likely to get it in people with T2DM compared to people who are not diabetic but are at risk and a control group that was not at risk in Sulaimani province, Iraq. A total of 160 subjects were enrolled, including 60 T2DM patients, 50 non-diabetic individuals with known risk factors, and 50 controls without diabetes or recognized exposure. Anti-*T. gondii* IgG and IgM antibodies were detected in serum samples using electrochemiluminescence immunoassay (ECLIA). *T. gondii* IgG antibodies were substantially more common in T2DM patients (66.7%) than in controls (10.0%) and at-risk non-diabetics (30.0%) ($P < 0.001$). When it came to environmental and lifestyle factors and their correlation with *T. gondii* infection, handling raw meat or unwashed vegetables was strongly linked to infection, although the source of drinking water was not. Female gender and older age were also significantly connected with infection. These results support a robust link between latent *T. gondii* infection and type 2 diabetes. In endemic areas, the prevalence of IgG antibodies suggests chronic infection rather than acute illness, underscoring the necessity of regular screening, health education, and focused interventions for high-risk populations, particularly women and older persons. To better understand the pathways relating to diabetes and toxoplasmosis, more study combining molecular and immunological investigation is advised.

Keywords: Electrochemiluminescence Immunoassay (ECLIA), Latent Toxoplasmosis, *Toxoplasma gondii* Seropositivity, Type 2 Diabetes Mellitus (T2DM).

1 INTRODUCTION

Humans are among the many warm-blooded creatures infected by *T. gondii*, an obligate intracellular protozoan parasite of the phylum Apicomplexan. Approximately one-third of the world's population is thought to have been exposed to this parasite, with prevalence ranging from ~0.5% to ~87% depending on socioeconomic, climatic, and geographic factors [1]. Transmission to humans mainly occurs through ingestion of tissue cysts in undercooked meat or food, or via water contaminated with oocysts excreted by infected cats, the definitive hosts. Less frequently, infection may result from blood transfusion, organ transplantation, or congenital transmission. Primary infection in immunocompetent individuals is often asymptomatic or produces mild flu-like symptoms, but *T. gondii* establishes lifelong latency within host tissues. In immunocompromised populations including patients with diabetes mellitus, cancer, HIV/AIDS, or organ transplant recipient's reactivation of latent infection may cause severe disease [2]. Type 2 diabetes mellitus (T2DM) is a major global health concern, projected to affect more than 500 million people by 2030. Approximately 90% of diabetes cases are type 2, which is characterized by progressive pancreatic β-cell dysfunction and insulin resistance [3,4]. Chronic hyperglycemia and low-grade systemic inflammation compromise immune function in T2DM patients, rendering them more vulnerable to opportunistic infections. Several studies have proposed a potential link between *T. gondii* infection

*Corresponding author: latif.mohammed@univsul.edu.iq

and T2DM, supported by both epidemiological evidence and emerging insights into molecular and immunological mechanisms [5]. Recent advances suggest that *T. gondii* infection may contribute to T2DM through immune regulatory dysfunction. For example, co-infection in diabetic patients has been shown to alter serum cytokine profiles, with increased TGF-β and decreased IL-6 levels, indicating parasite-driven modulation of host immunity [8]. Similarly, *T. gondii* infection has been associated with altered expression of PD-1/PD-L1 immune checkpoint molecules, which may impair immune surveillance in diabetics [9]. Another mechanism involves host metabolic reprogramming. A recent study showed that *T. gondii* infection drives cholesterol metabolic remodeling in host cells by suppressing synthesis enzymes, reducing LDLR-mediated uptake, and upregulating the cholesterol oxidation pathway via CH25H, producing immunomodulatory 25-hydroxycholesterol [10]. Such metabolic disruptions are strongly implicated in insulin resistance and β-cell dysfunction. Moreover, *T. gondii* infection can induce ER stress and unfolded protein response (UPR), pathways linked to insulin resistance and β-cell failure in diabetes. Though human data remain limited, experimental evidence highlights the potential for parasite-induced UPR to exacerbate diabetic pathology [11]. Genotypic variations of the parasite may also influence disease outcomes. A study from Iran employing nested-PCR and sequencing found that *T. gondii* genotype III was present among diabetic patients, suggesting that strain-specific effects could modulate pathogenicity and host immune responses [12]. At the population level, epidemiological evidence consistently demonstrates higher *T. gondii* seroprevalence in T2DM patients compared to controls. For instance, in Khuzestan province, Iran, seropositivity among diabetics was ~44.3% versus ~19% in non-diabetic individuals [13]. Likewise, in Minia, Egypt, T2DM patients exhibited significantly higher *T. gondii* seropositivity, and infection was linked to diabetic complications [14]. A systematic review and meta-analysis of 25 studies further confirmed that individuals with *T. gondii* infection had nearly 2.77 times higher odds of developing T2DM [15]. In Iraq, prior studies have reported variable seroprevalence of *T. gondii* among diabetic populations, with some suggesting significant associations and others finding weaker correlations [6,7]. However, data from the Kurdistan Region remain scarce. Therefore, the present study aimed to determine the seroprevalence of *T. gondii* infection among T2DM patients in Sulaimani Province and to assess lifestyle and sociodemographic factors associated with infection. It is anticipated that these findings will provide valuable insights into the epidemiological and biological links between diabetes and toxoplasmosis, contributing to improved prevention strategies for high-risk groups.

2 MATERIALS AND METHODS

Between December 8, 2024, and April 7, 2025 a cross-sectional study was carried out in Sulaimani Province, in the Kurdistan Region of Iraq. Three groups were the focus of the study: people with type 2 diabetes mellitus (T2DM), people without diabetes who had known risk factors, and people who appeared to be in good health. To guarantee a representative sample, participants were gathered from community health clinics, diabetic centers, and hospitals. The study comprised 160 participants in total: 60 patients with type 2 diabetes mellitus (T2DM), 50 healthy controls, and 50 non-diabetic people with risk factors. 160 participants in all were enrolled, including 60 clinically diagnosed T2DM patients (The diagnosis of T2DM among patients was confirmed based on their clinical records obtained from the Diabetes and Endocrine Center, as documented by the attending physicians),50 non-diabetic people who were thought to be at risk of infection because of their lifestyle, and 50 healthy people without diabetes and without any known risk factors.

The T2DM group had to be at least 30 years old, have a verified diagnosis of T2DM, and be prepared to give informed permission. People without diabetes who had a history of handling raw meat or unclean vegetables or regular animal interaction were included in the at-risk category. Healthy adults without diabetes or certain toxoplasmosis risk factors made up the control group.

About 5 milliliters of venous blood were drawn aseptically from each participant. Before being analyzed in the laboratory, part of the laboratory work was conducted at the private Bazyan Laboratory, while the others were performed in the Research Laboratory of the College of Medicine, University of Sulaimani, the serum was kept at -40 °C for up to three months after being separated by centrifugation at 3000 rpm for ten minutes. Following the manufacturer's instructions, a commercial electrochemiluminescence immunoassay (ECLIA) kit was used on the Cobas e 411 analyzer (Roche Diagnostics, Japan) to detect the existence of anti-*T. gondii* IgG and IgM antibodies. The sandwich concept that underpins this technique enables the quantitative and qualitative detection of IgG and IgM antibodies. The kit's internal controls were applied to guarantee the precision and dependability of the findings.

A systematic questionnaire was also used to gather clinical history, demographic information (e.g., age, gender, education, and residency, contact with animals, handling raw meat and unwashed vegetables, fast food consuming, and source of drinking water), and details on possible risk factors, such as dietary practices, Pregnant women, individuals with a history of animal breeding, vegetable sellers, and participants with other chronic disorders (in addition to T2DM) were also included, as they represent important exposure groups. Participants with known immunocompromised conditions (e.g., HIV infection, cancer, organ transplantation…etc.) were excluded from the study.

STATISTICAL ANALYSIS

Version 26 of the SPSS software was used to analyze the data. Clinical and demographic features were gathered using descriptive statistics. Potential risk factors for *T. gondii* infection were assessed using logistic regression analysis, and relationships between categorical variables were evaluated using the Chi-square test. P-values less than 0.05 were regarded as statistically significant.

3 RESULTS

A total of 160 participants were included in the study: 60 patients with type 2 diabetes mellitus (T2DM), 50 non-diabetic individuals with known risk factors for T. gondii infection, and 50 healthy controls (HC). The prevalence of Toxoplasma gondii IgG antibodies were highest in the T2DM group (66.7%), followed by the non-DM group (30.0%), and lowest in the HC group (10.0%). Consequently, the proportion of participants testing negative for IgG was 33.3% in the T2DM group, 70.0% in the non-DM group, and 90.0% in the HC group. Statistical analysis showed significant differences between groups: HC vs Non-DM ($P = 0.012$), HC vs T2DM ($P < 0.001$), and non-DM vs T2DM ($P < 0.001$), indicating a clear trend toward higher T. gondii seroprevalence among individuals with T2DM. These results are summarized in (Table 1, Figure 1).

Table 1. Prevalence of anti- *T. gondii* IgG antibodies across study groups.

IgG	Studied Groups				P value		
	HC (No at risk) N=50(%)	Non-DM (At risk) N=50(%)	DMT2 N=60(%)	Total N=160(%)	Pa	Pb	Pc
Negative	45 (90.0)	35 (70.0)	20 (33.3)	100 (62.5)			
Positive	5 (10.0)	15 (30.0)	40 (66.7)	60 (37.5)	0.012 *	<0.001 **	<0.001 **
total	36 (72.0)	30 (60.0)	33 (55.0)	99 (61.9)			

N: Number; %: Percentage. The P-values represent the significance of comparisons between the groups, with each group being compared as follows: Pa compares Group 1 (HC) with Group 2 (Non-DM), Pb compares Group 1 (HC) with Group 3 (DMT2), and Pc compares Group 2 (Non-DM) with Group 3 (DMT2). The P-values indicate the level of statistical using Chi square test.

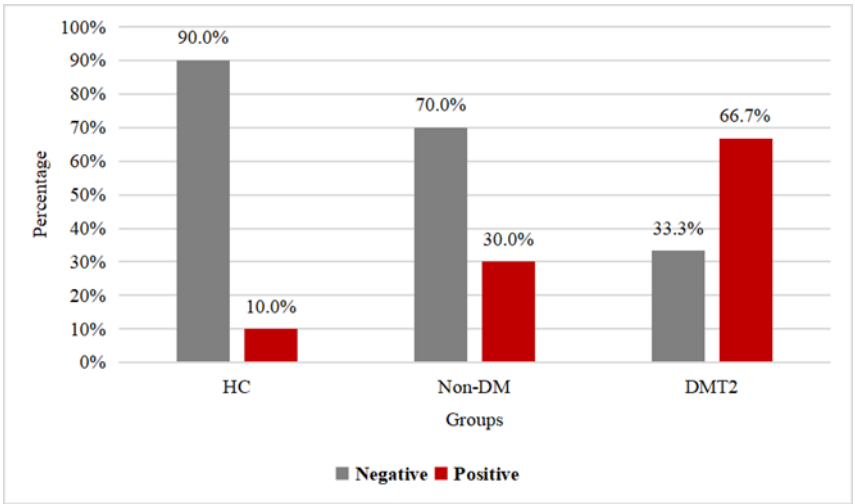

FIGURE 1. Prevalence of anti- *T. gondii* IgG antibodies across study groups.

All participants in the three study groups (T2DM, Non-DM at-risk, and HC) tested negative for anti-T. gondii IgM antibodies. This indicates that no acute infections were detected during the study period.

Association between *T. gondii* seroprevalence and gender. Females showed a significantly higher rate of IgG positivity (71.7%) compared to males (28.3%), with a statistically significant P-value of 0.007. This suggests that females may have a higher likelihood of *T. gondii* infection in the studied population (Table 2).

Table 2. Gender distribution of anti-*T. gondii* IgG seropositivity among study participants.

Sociodemographic factors	IgG			P value
	Negative N=100, 62.5%	Positive N=60, 37.5%	Total N= 160, 100.0%	
Gender				
Female	50 (50.0)	43 (71.7)	93 (58.1)	0.007 *
Male	50 (50.0)	17 (28.3)	67 (41.9)	

Table 3 demonstrates the prevalence of IgG positivity varied significantly across age groups. Among individuals aged ≤ 40 years, only 21.7% revealed positive for *T. gondii*, while the prevalence increased with age. In those aged 51-60 years, 30.0% revealed positive reactions for anti *T. gondii* IgG antibodies, and in individuals aged >60 years, the positivity rate reached 31.7%. The significant difference in IgG positivity between age groups (P = 0.023).

Table 3. Age distribution of anti-*T. gondii* IgG seropositivity among study participants.

Sociodemographic factors	IgG			P value
	Negative N=100, 62.5%	Positive N=60, 37.5%	Total N= 160, 100.0%	
Age (year)				
≤ 40	44 (44.0)	13 (21.7)	57 (35.6)	0.023 *
41-50	17 (17.0)	10 (16.7)	27 (16.9)	
51-60	17 (17.0)	18 (30.0)	35 (21.9)	
>60	22 (22.0)	19 (31.7)	41 (25.6)	

LIFESTYLE FACTORS AND THEIR ASSOCIATION WITH T. GONDII INFECTION

Regarding drinking water source, the majority of participants across all groups were consuming tap water (79.4%). Specifically, 76.7% of individuals consuming tap water tested IgG positive, whereas filtered water and well water consumption showed lower rates of IgG positivity at 8.3% and 15.0%, respectively. However, despite these variations, the differences in IgG positivity across the different water sources were not statistically significant, as indicated by the P-value of 0.132. In contrast, a significant association was found between handling raw meat or unwashed vegetables and IgG positivity. Among individuals who reported handling raw meat or unwashed vegetables, 73.3% tested IgG positive, compared to only 26.7% of those who did not handle raw meat or unwashed vegetables. The P-value of 0. 021.As shown in (Table 4).

Table 4. Association of anti-*T. gondii* IgG seropositivity with source of drinking water, and handling raw meat or unwashed vegetables.

Lifestyle Factors	IgG		Total N= 160, 100.0%	P value
	Negative N=100, 62.5%	Positive N=60, 37.5%		
Source of drinking water				
Tab water	81 (81.0)	46 (76.7)	127 (79.4)	0.132 ns
Filtered water	13 (13.0)	5 (8.3)	18 (11.3)	
Well water	6 (6.0)	9 (15.0)	15 (9.4)	
Handling raw meat or unwashed vegetables				
No	45 (45.0)	16 (26.7)	61 (38.1)	0.021 *
Yes	55 (55.0)	44 (73.3)	99 (61.9)	

4 DISCUSSION

The current study evaluated the seroprevalence of *T. gondii* in Sulaimani Province, Iraq, among patients with type 2 diabetes mellitus (T2DM), healthy controls, and non-diabetic at-risk individuals. The results showed that *T. gondii* IgG antibodies were substantially more common in T2DM patients (66.7%) than in at-risk non-diabetics (30.0%) and healthy controls (10.0%). This agrees with previous Iraqi studies from Baghdad and Kirkuk, which also reported significantly higher infection rates in diabetic patients compared with controls [21]. Likewise, studies from Iran [24,26] and Egypt [25] found higher seroprevalence in diabetic groups, indicating that our results are in line with both local and international findings. However, a few reports from other regions failed to establish a significant relationship [23], suggesting that differences in methodology, population size, or local epidemiological conditions may influence outcomes.

In fact, all study participants tested negative for IgM antibodies, confirming the absence of acute toxoplasmosis. This aligns with the epidemiological pattern observed in many endemic regions, where latent IgG seropositivity dominates and acute IgM positivity is relatively rare. Our findings are in agreement with previous Iraqi and regional studies that similarly reported very low [28] or absent IgM detection among diabetic populations [29]. This is consistent with the global epidemiology of toxoplasmosis, where infections often remain subclinical but persist lifelong. Also, our findings are comparable to those from Iran [24] and Egypt [25], where IgG was similarly dominant in diabetic cohorts, reinforcing that latent toxoplasmosis is the main burden among diabetic populations.

The significant association between female gender and higher seropositivity in this study is also in agreement with earlier Iraqi and Middle Eastern reports [16,25]. Possible explanations include greater female involvement in household and food-related tasks, which may increase exposure. Similar patterns were documented in Minia, Egypt, where women were at greater risk of infection than men [25]. Thus, our results are consistent with these observations, although studies in some Western countries have reported no gender difference, possibly due to different cultural roles.

Age was another significant factor, with older participants showing higher infection rates. This agrees with Iraqi [17], Iranian [18,24], and Turkish [19] studies, which all documented age-related increases, reflecting cumulative exposure risk. No disagreement was detected here, as most regional studies consistently report age as a key determinant.

Our study found no significant association between drinking water source and *T. gondii* seropositivity, despite higher proportions among well-water users. This partially contrasts with studies in Iran and Egypt [20,25], where contaminated water was a significant transmission route. The discrepancy may be due to our smaller number of well-water consumers or more effective water treatment in the study area. Therefore, while our data point in the same direction, the lack of significance highlights the need for larger sample sizes.

By contrast, the association between handling raw meat/unwashed vegetables and seropositivity ($p = 0.021$) strongly agrees with earlier Iraqi [8], Iranian [18,24], Turkish [19], and Egyptian [25] findings, all of which identified undercooked meat and raw produce as major risk factors. This agreement underscores the importance of food safety in controlling toxoplasmosis, especially among vulnerable groups such as diabetics.

Our prevalence (66.7% in diabetics) falls within the range reported globally. For example, the rate is slightly higher than that in Iran's Khuzestan province (44.3%) [24] and similar to Khorramshahr (72.5%) [26]. It is also close to the Egyptian Minia study, which reported ~74% in diabetics [25]. Thus, our findings generally agree with the higher end of the prevalence spectrum, consistent with Iraq being a highly endemic region. On the other hand, a recent systematic review reported a pooled global OR of 2.77 for the association between *T. gondii* and T2DM [23], which is lower than the odds suggested by our results. This suggests that Iraqi diabetics may face an even greater risk, perhaps due to regional factors such as dietary practices and close animal contact.

Finally, an Iraqi study linked *T. gondii* seropositivity with overweight and obesity in diabetics [27]. While we did not analyze BMI, this supports the idea that toxoplasmosis may interact with metabolic abnormalities, a finding consistent with our observation of higher prevalence in diabetics. Future local studies should therefore explore the relationship between parasite infection, obesity, and diabetes control.

The biological mechanisms underlying the observed association remain to be fully elucidated. It is hypothesized that chronic toxoplasmosis may contribute to systemic inflammation and oxidative stress, thereby aggravating metabolic disturbances in diabetes. Conversely, T2DM may predispose patients to toxoplasmosis due to impaired immune function, particularly in cell-mediated responses that are essential for controlling *T. gondii*. Future studies integrating immunological and molecular approaches are required to clarify the direction and causality of this relationship.

This study has several strengths, including the use of a reliable electrochemiluminescence immunoassay (ECLIA) method for antibody detection and the inclusion of both diabetic and non-diabetic groups, which allows comparison of relative risks. However, limitations should also be acknowledged. The sample size was modest, and the study was restricted to a single geographic area, which may limit generalizability. Additionally, cross-sectional design precludes inference of causality.

Overall, the results highlight the importance of considering toxoplasmosis in diabetic populations, particularly in endemic regions such as Iraq. Screening and preventive health education may reduce risks associated with latent infection in vulnerable groups.

CONCLUSION

In Sulaimani Province, Iraq, this study showed that patients with type 2 diabetes mellitus had a considerably greater frequency of *T. gondii* infection than both healthy controls and non-diabetic at-risk persons. While the source of drinking water had no discernible effect, touching raw meat and unwashed vegetables, being a woman, and being older were all strongly linked to infection. All cases represented chronic latent infections rather than acute illnesses, as shown by the preponderance of IgG antibodies. These results imply that latent infection may further exacerbate the health state of diabetics and that type 2 diabetes may enhance susceptibility to toxoplasmosis.

The findings offer epidemiological proof that parasitic screening should be incorporated into diabetes care plans, particularly in areas where toxoplasmosis is more prevalent. In order to evaluate the possible clinical consequences of chronic toxoplasmosis in diabetes patients and to look into the immunological processes behind the observed correlation, more research is necessary.

ETHICAL APPROVAL

The University of Sulaimani, College of Medicine's Ethical Committee granted ethical approval for this study (Code: 24, Date: 13/10/2024). Before being enrolled, each subject gave their informed consent. Throughout the whole trial, participant data was kept private.

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CONFLICTS OF INTEREST

The author declares no conflict of interest.

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