Is Toxoplasmosis A Risk Factor in Diabetic Patients in Tehran?

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**Article Summary**

**Aims:** Toxoplasmosis is a cosmopolitan zoonotic disease caused by an obligate apicomplexan intracellular parasite known as *Toxoplasma gondii* (*T. gondii*). Recently, toxoplasmosis has been suggested as a risk factor for diabetes. Thus, the present study aimed to assess the association between *T. gondii* infection and two types of diabetes in Tehran, the capital of Iran.

**Materials & Methods:** In the current cross-sectional study, 98, 95, and 94 blood samples were collected from Type 1 and Type 2 diabetic and nondiabetic individuals, referring to Imam Sajad hospital from February to August 2018, respectively. Anti-*T. gondii* specific IgG and IgM antibodies were measured using enzyme-linked immunosorbent assay (ELISA). Moreover, a structured demographic questionnaire was completed for each person.

**Results:** IgG antibody was found to be positive in 16.32 (16 of 98) and 57.89% (55 of 95) of patients with diabetes Type 1 and Type 2 and 17.02% (16 of 94) of nondiabetic individuals as controls, respectively. However, the prevalence of positive IgM antibody in these groups was determined as 2.04 (2 of 98), 6.32 (6 of 95), and 17.02 % (16 of 94), respectively.

**Conclusion:** This finding revealed that toxoplasmosis could be considered as a possible risk factor for diabetes Type 2, while no statistically significant association was found between *T. gondii* infection and diabetes Type 1. More research is required to be conducted in the future in order to better understand this association.

**Keywords:** *Toxoplasma gondii*, Diabetes, Frequency, Tehran.

**Citation Links**

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34. Toxoplasmosis, pancreatitis, obesity and drug...

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Introduction
Toxoplasmosis is a ubiquitous parasitic zoonosis caused by a protist with intracellular localization, called *Toxoplasma gondii* (*T. gondii*) which infects a diverse spectrum of warm blooded animal species, including humans [1-2]. As a public health concern, one-third of the human population is estimated to be infected by this protozoan [3]. In addition, this parasite is a thriving microorganism by virtue of great compatibility to diverse host species and employing various transmission routes comprising oocyst-contaminated food/water, cyst-infected raw meat, vertical transmission, organ transplantation, and transfusion of contaminated blood supplies [4]. On an international scale, annually 190,100 (95% CI = 179,300 – 206,300) cases are congenitally infected with *Toxoplasma* infection, predominantly in South America and some Middle Eastern nations [5]. In developing and industrialized countries, the seroprevalence rates of 60 and 30% have been reported for *Toxoplasma* infection, respectively [6]. In Iran, the overall seroprevalence rate of toxoplasmosis among general population has been reported to be 39.3% (95% CI = 33.0% - 45.7%) [3]. While toxoplasmosis is usually asymptomatic in healthy individuals, neurological complications and congenital abnormalities (abortion, stillbirth, hydrocephalus, and blindness) may be anticipated for toxoplasmosis in pregnant women and immunocompromised individuals in particular [7-8].

Diabetes mellitus (DM) is considered as a chronic metabolic disorder, which is of particular importance in people on high-calorie diets. Insulin hormone secretion dysfunction (Type 1 diabetes, T1DM) and/or disoriented response to insulin by target cells (Type 2 diabetes, T2DM) provoke this unhealthy condition leading to hazardous hyperglycemia with harsh sequelae [9]. DM is to a greater extent premised to be related to lifestyle, habits, genetic background, and autoimmune processes, reaching approximately 522 million morbidity rate by 2030. According to the previous reports, there exists probably a relationship between DM and infectious pathogens such as Coxsakie B4 virus [10] and *Helicobacter pylori* [11]. In this context, *T. gondii* may also have a role in DM causality, and various studies are now focused on this aspect around the world [12-18].

**Objectives:** This study intended to investigate the seroprevalence of *T. gondii* infection among T1DM and T2DM patients referring to Imam Sajjad hospital, Tehran, Iran using enzyme-linked immunosorbent assay (ELISA).

**Material and methods**

**Study area and study design:** Tehran is the capital and most crowded city of Iran with approximately 8.7 million residents and 15 million metropolitan population, located in an area of around 574 km². A cold semi-arid continental climate with a Mediterranean precipitation pattern is predominant in Tehran, defined by Alborz mountain ranges to the north and central desert to the south. In general, Tehran possess mild spring and autumn, dry and hot summer as well as wet and cold winter. Throughout the year, there is an average of 429 mm precipitation and 46% relative humidity (https://en.wikipedia.org/wiki/Tehran).

This case-control study aimed to evaluate the association between *T. gondii* infection and T1DM and T2DM patients referring to Imam Sajjad Hospital in Tehran, Iran using ELISA serological technique.
Questionnaire: A questionnaire was designed to involve some probable risk factors for toxoplasmosis and to assess their impact on the outcomes of examined individuals. Demographic parameters and risk factors included in the questionnaire were as follows: age, gender, education, animal contact, history of serious disease, and DM type (T1DM and T2DM).

Participants and sampling: Totally, 287 participants were randomly included in this study, who referred to Imam Sajjad Hospital in Tehran from February to August 2018. After obtaining consent form of each person to secure the trust and confidence, a pre-designed questionnaire was filled out, then 3 mL clotted blood (for sera samples) and 2 mL EDTA-incorporated blood (for HbA1C test) were collected of each person. Diabetic patients were those with positive fasting blood glucose and HbA1C (glycated hemoglobin, indicating 3-months blood glucose), while control individuals were negative. Sera were obtained by centrifugation (3000 rpm, 10 min) and kept frozen at -20 °C for further use.

ELISA test: In order to discern the acute and chronic phases of the infection, anti-*T. gondii* IgM and IgG antibodies titers were detected in obtained sera using two commercial capture ELISA kits, Captia™ Toxoplasma gondii IgM and IgG (Trinity Biotech, Ireland), according to the manufacturer’s instructions.

Statistical analysis: SPSS software (Ver. 21) was used for results analysis with a p-value < 0.05 as statistically significant. To compare the seroprevalence values with sociodemographic data and risk factors of included subjects, chi-squared and fisher’s exact tests were employed.

Findings
In total, 98 T1DM and 95 T2DM as well as 94 control subjects enrolled in this investigation. IgG antibody was found to be positive in 16.32 (16 of 98) and 57.89% (55 of 95) of patients with diabetes Type 1 and Type 2 and 17.02% (16/94) of nondiabetic individuals as controls. However, the prevalence of positive IgM antibody in these groups was determined as 2.04 (2 of 98), 6.32 (6 of 95), and 17.02 % (16 of 94), respectively.

According to ELISA test results, 2.04 and 6.32% of T1DM and T2DM patients and 17.02% of control individuals had anti-*Toxoplasma* specific IgM antibody, respectively. The IgM seroprevalence was statistically significant among the T1DM (p < .0001) and T2DM (p = .022) patients compared to the control group (Table 1, Fig. 1). Moreover, 16.32 and 57.89% of T1DM and T2DM patients and 17.02% of control subjects were positive for anti-*T. gondii* specific IgG, respectively. This seropositivity was statistically significant among the T2DM patients in comparison with T1DM (p < .0001) or control groups (p < .0001). Also, the comparison of IgG in DM patients was statistically significant (p < .0001) (Table 2, fig. 2).

The details of sociodemographic parameters and risk factors analysis for T1DM patients and control individuals are shown in Table 3. The age of the examined patients was a significant parameter (p = .007), and the highest seroprevalence of Toxoplasma infection was observed in T1DM patients in the age group of 20-30 years. There was a significant difference between the seropositive male (14%) and female (60%) individuals (p = .007). Additionally, there was no significant difference in seroprevalence of Toxoplasma infection regarding education, animal contact, and history of serious disease. Regarding the evaluated factors for T2DM patients, there was no statistically significant difference in seroprevalence of
Table 1) Frequency of anti-Toxoplasma IgM among T1DM & T2DM groups

<table>
<thead>
<tr>
<th></th>
<th>T1DM (n = 98)</th>
<th>T2DM (n = 95)</th>
<th>Control (n = 94)</th>
<th>x²</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Toxoplasma IgM +ve</td>
<td>2 (2.04)</td>
<td>6 (6.32)</td>
<td>16 (17.02)</td>
<td>x²=14.827</td>
<td>P=.001</td>
</tr>
<tr>
<td>Anti-Toxoplasma IgM -ve</td>
<td>96 (97.96)</td>
<td>89 (93.68)</td>
<td>78 (82.98)</td>
<td>x²=5.265</td>
<td>P=.022</td>
</tr>
</tbody>
</table>

p*: T1DM vs T2DM, p**: T1DM vs Control, p***: T2DM vs Control

Table 2) Frequency of anti-Toxoplasma IgG among T1DM & T2DM groups

<table>
<thead>
<tr>
<th></th>
<th>T1DM (n= 98)</th>
<th>T2DM (n= 95)</th>
<th>Control (n= 94)</th>
<th>x²</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Toxoplasma IgG +ve</td>
<td>16 (16.32)</td>
<td>55 (57.9)</td>
<td>16 (17)</td>
<td>x²=35.844</td>
<td>P*.000</td>
</tr>
<tr>
<td>Anti-Toxoplasma IgG -ve</td>
<td>82 (83.7)</td>
<td>40 (42.1)</td>
<td>78 (83)</td>
<td>x²=0.017</td>
<td>P**=.897</td>
</tr>
</tbody>
</table>

p*: T1DM vs T2DM, p**: T1DM vs Control, p***: T2DM vs Control

Figure 1) Frequency of anti-Toxoplasma IgM among T1DM & T2DM groups

Figure 2) Frequency of anti-Toxoplasma IgG among T1DM & T2DM groups
Table 3) Frequency of T. gondii IgG in T1DM patients and controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>T1DM (N= 98)</th>
<th>Controls (N= 94)</th>
<th>T1DM vs Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>No. positive</td>
<td>%</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>44</td>
<td>4</td>
<td>9.1</td>
</tr>
<tr>
<td>20- 30</td>
<td>41</td>
<td>8</td>
<td>19.5</td>
</tr>
<tr>
<td>31- 40</td>
<td>11</td>
<td>2</td>
<td>18.2</td>
</tr>
<tr>
<td>&gt;41</td>
<td>2</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>93</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate &amp; primary school</td>
<td>17</td>
<td>4</td>
<td>23.5</td>
</tr>
<tr>
<td>Diploma &amp; post diploma</td>
<td>54</td>
<td>6</td>
<td>11.1</td>
</tr>
<tr>
<td>Higher education (BSc or higher)</td>
<td>27</td>
<td>6</td>
<td>22.2</td>
</tr>
<tr>
<td>Animal contact</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>1</td>
<td>7.1</td>
</tr>
<tr>
<td>No</td>
<td>84</td>
<td>15</td>
<td>17.9</td>
</tr>
<tr>
<td>History of other diseases</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>21</td>
<td>1</td>
<td>4.8</td>
</tr>
<tr>
<td>No</td>
<td>77</td>
<td>15</td>
<td>19.5</td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td>16</td>
<td>16.3</td>
</tr>
</tbody>
</table>
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Table 4) Frequency of T. gondii IgG in T2DM patients and controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>T2DM (N= 95)</th>
<th>Controls (N= 94)</th>
<th>T2DM vs Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N0. tested</td>
<td>No. positive</td>
<td>%</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20- 30</td>
<td>6</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>31- 40</td>
<td>6</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>&gt;41</td>
<td>80</td>
<td>49</td>
<td>61.3</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>57</td>
<td>30</td>
<td>52.6</td>
</tr>
<tr>
<td>Female</td>
<td>38</td>
<td>25</td>
<td>65.8</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate &amp; primary school</td>
<td>35</td>
<td>22</td>
<td>62.9</td>
</tr>
<tr>
<td>Diploma &amp; post diploma</td>
<td>39</td>
<td>18</td>
<td>46.2</td>
</tr>
<tr>
<td>Higher education (BSc or higher)</td>
<td>21</td>
<td>15</td>
<td>71.4</td>
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<td><strong>Animal contact</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15</td>
<td>8</td>
<td>53.3</td>
</tr>
<tr>
<td>No</td>
<td>80</td>
<td>47</td>
<td>58.8</td>
</tr>
<tr>
<td><strong>History of other diseases</strong></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>33</td>
<td>15</td>
<td>45.5</td>
</tr>
<tr>
<td>No</td>
<td>62</td>
<td>40</td>
<td>64.5</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
<td>55</td>
<td>57.9</td>
</tr>
</tbody>
</table>
Toxoplasma infection (Table 4).

Discussion

Despite passing a century since its discovery, *T. gondii* protist has still remained as a mystery because many aspects of its biology and pathogenesis are yet to be elucidated [19]. The facts that *Toxoplasma* could eventually propagate in any nucleated host cell and that cell-mediated immune responses as well as antibody production are substantially subdued during chronic toxoplasmosis, have raised this question that this protozoan parasite may have an underlying role in triggering, accelerating, or precipitating the mechanistic processes of overt clinical autoimmunity conditions such as DM [17, 20].

In recent years, there have been increasing works designed as cross-sectional or case-control studies on the association between *T. gondii* infection and the risk of DM occurrence with conflicting findings. Interestingly, most of such investigations have been conducted in some Middle Eastern countries such as Iran, Iraq, and Egypt, while there exists a huge gap in data in other parts of the globe [20-21]. In current case-control study, it was attempted to address such association in T1DM and T2DM patients referring to Imam Sajjad hospital in Tehran using ELISA method.

Overall, 16 out of 98 T1DM and 55 out of 95 T2DM patients and 16 out of 94 control individuals were found to be infected by *T. gondii*, which was statistically significant among the all groups (*p* < .0001). Such finding is consistent with the findings of some studies by Shirbazou et al. (2013) (*p* = .001) [22], Jafari Modrek et al. (2015) (*p* = .015) [23], Saki et al. (2016) (*p* < .05) [24] in Iran, Hemida et al. (2018) (*p* = .03) [25], and Beshay et al. (2018) (*p* = .027) [12] in Egypt. Nevertheless, some studies results counteract with this study results; for instance, Khalili et al. (2018) and Siyadatpanah et al. (2013) in Iran indicated no statistically significant difference between DM and nondiabetic groups [26-27]. Additionally, another case-control study in Mexico revealed no association between *Toxoplasma* infection and DM (*p* = .18) [28].

Precisely, the present study results showed that only T2DM patients had considerably elevated levels of anti-*Toxoplasma* specific IgG titers (57.9%) in comparison with the control group (17%), which was statistically significant (*p* < .0001). Similarly, Younis et al. (2018) reported a high *Toxoplasma* seroprevalence (41.5%) in T2DM patients compared to the control subjects (24%) [29]. Furthermore, Li et al. (2018) in China represented significantly higher *Toxoplasma* seroprevalence in T2DM patients (23.5%) than in the control group (11.75%) (*p* < .001) [30]. Two recent meta-analysis studies also emphasized the present study findings and remarked a positive association between *Toxoplasma* exposure and T2DM occurrence, while Majidiani et al. (2018) rejected such probable relevance regarding T1DM [20-21].

Beyond the last two decades, direct and indirect links between inflammation and T2DM have been clarified, suggesting this type of DM as a chronic inflammatory disease [17, 31-32]. For instance, T2DM progression has been increasingly correlated with β-cells dysfunction and/or necrosis through reactive oxygen species (ROS), nitric oxide (NO), and various pro-inflammatory cytokines, leading to possible insulin resistance in animals [17, 20, 33]. In such molecular milieu induced by T2DM, latent *T. gondii* cysts may be reactivated and cause infection. Furthermore, efficient intracellular replication of *Toxoplasma* tachyzoites has been elucidated in the presence of D-glucose and insulin in a dose-response manner [34]. Altering inflammatory fat distribution following *T. gondii* lodging in fatty tissues is proposed as a plausible initiation factor for obesity [35]. Since T2DM is highly associated with obesity, *Toxoplasma* infection could indirectly
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provoked this chronic disease. Besides, T. gondii-induced behavioral changes through dopamine pathway alternations, such as overeating, may accidentally promote obesity and subsequent T2DM. Recently, a case-control study on Chinese population showed that the risk of developing hypertension in T. gondii-infected T2DM patients is 2.34-fold more than those without Toxoplasma infection. All these data are pieces of a puzzle, which together imply the plausible central role of this parasitic infection in DM development and complications.

The seroprevalence of IgG in T1DM patients was 16.3% (p = .897), which is in agreement with the result (16.5%) obtained by Li et al. (2018) in China through a commercial ELISA test but disagreement with the result (5.4%) obtained by Krause et al. (2009) in Colombia via BioPlex 2200 as a novel flow cytometry technique. In contrary, Beshay et al. (2018) in Egypt utilized Electrochemiluminescence IgG immunoassay and determined a very high seroprevalence for IgG (86.37%) in T1DM patients. Also, two other Egyptian investigations using ELISA approach denoted higher seroprevalence rates for IgG than that reported in the present study, similar to Gokce et al.’s (2008) study in Turkey. Nevertheless, there was no statistically significant association between T1DM and T. gondii positive individuals (p = .897), in consistent with other studies results. Regarding sociodemographic parameters and risk factors examined in this study, there was no statistically significant difference between T2DM cases. Consistent with the present study, gender wasn’t a significant parameter (p = .63) in T2DM patients in Libya; although age was a significant parameter (p = .013) with the most positive cases in the age group of 29-39 years. Li et al. (2018) in China also reported other significant risk factors for T2DM cases, including keeping cats at home (p = .017), consumption of raw/undercooked meat (p < .001), consumption of oyster (p < .001), and consumption of fish (p < .001). Regarding T1DM patients in this study, only age (P = 0.007) and gender (P = 0.007) were statistically significant parameters, and the age group of 20-30 years and men were among the most affected patients, respectively. In agreement with the present study result, only age was a significant parameter in a study in Iraq (p = .003). In addition, Khattab et al. (2019) reported other risk factors consisting of residence (p < .001), undercooked meat (P = 0.001), and contact with cat and soil (p < .0001).

This study met some limitations as follows: 1) not considering more risk factors for this case-control study such as consumption of raw/undercooked meat and obesity/overweight; 2) lack of standard criteria for various parameters such as T2DM diagnosis (methodological heterogeneity); and 3) not excluding patients suffering from psychiatric illnesses because such individuals cause a clinical heterogeneity concern and are mostly seropositive for T. gondii. Moreover, there is a need for more studies to evaluate stage-specific serum inflammatory biomarkers in order to more shed light on the association between the T2DM-Toxoplasma paradigm. In addition, nationwide case-control sero-surveys are required in all age groups by considering all likely confounding factors. In conclusion, this study indicated that the frequency of anti-Toxoplasma specific IgG antibodies was more in T2DM patients as compared to the control group.

**Conclusion**

This finding revealed that toxoplasmosis is as a possible risk factor for diabetes type 2, while no statistically significant association was found between T. gondii infection and diabetes type 1. More research is required.
to better understanding of this association in the future.

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**Ethical permissions:** This study was approved by the ethical committee of Tarbiat Modares University (ID: IR.MODARES.REC.1397.179).

**Conflicts of interests:** The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

**Authors’ Contribution:** Shamsinia S. and Dalimi A. conceived the study and designed the study protocol; Dalimi A. was the supervisor; M. Pirestani was the advisors and Shamsinia S. was the MSc student of this study. All authors read and approved the final version of the manuscript.

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