The Probable Association between Chronic *Toxoplasma gondii* Infection and Type 1 and Type 2 Diabetes Mellitus: A Case-Control Study

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**Purpose.** The probable association between *Toxoplasma gondii* (*T. gondii*) infection and diabetes mellitus (DM) is still controversial, and there are several studies with conflicting results. Thus, this study was performed to assess the possible association between chronic *T. gondii* infection and type 1 diabetes mellitus (T1DM) and T2DM.

**Methods.** In this case-control study, a total of 105 diabetic subjects including 36 patients with T1DM and 69 patients with T2DM were recruited. In addition, 150 nondiabetic subjects were enrolled as controls. Each case group had its own control group. Each participant completed a structured questionnaire obtaining demographic information. Serum samples were examined for *T. gondii*-specific IgG antibody using enzyme-linked immunosorbent assay (ELISA) method.

**Results.** Analysis revealed that 69.4% and 34.0% of patients with T1DM and control subjects were serologically positive for *T. gondii*, respectively (odds ratio (OR): 4.41; 95% confidence interval (CI): 1.75–11.06; \( P = 0.001 \)). Moreover, 72.5% of T2DM patients and 29.0% of healthy individuals were seropositive for *T. gondii* (OR: 6.44; 95% CI: 3.25–12.74; \( P < 0.001 \)). Among risk factors, only contact with cats was significantly associated with IgG seroprevalence in both T2DM patients (\( P < 0.001 \)) and control subjects (\( P = 0.045 \)).

**Conclusion.** Although the results showed that chronic *T. gondii* infection is significantly associated with T1DM and T2DM, there remain many questions regarding the exact mechanisms of *T. gondii* in the pathogenesis of DM.

**1. Introduction**

*Toxoplasma gondii* (*T. gondii*) is an obligate apicomplexan intracellular parasite that is capable of infecting nearly all warm-blooded animal species, including humans [1]. There are various routes of *T. gondii* transmission to humans: ingestion of oocyst-contaminated food or water, eating cyst-infected raw meat, vertical transmission from mother to fetus, organ transplantation, and blood transfusion [2–5]. It is estimated that one-third of the human population worldwide are infected with this parasite [2, 6, 7]. Previous systematic review articles in Iran have reported high *T. gondii* seroprevalence rates of more than 45% in various human groups, including HIV/AIDS patients, cancer patients, transplant recipients, and hemodialysis patients when compared to lower seroprevalence rates observed in the general population including healthy blood donors and pregnant women [8, 9].

Diabetes mellitus (DM) is one of the major worldwide public health concerns of the 21st century. It is estimated that the number of persons suffering from DM will increase to 552 million (7.7%) in 2030 [10]. Diabetic patients have suppressed immune systems, potentially indicating that these subjects may be more susceptible to acquire *T. gondii* infection.
interdisciplinary perspectives on infectious diseases

2. Materials and Methods

2.1. Study Area. The study was carried out in Khorramshahr city (Khuzestan province, southwest Iran, 30.4256°N, 48.1891°E) (Figure 1). At the 2016 census, its population was 170,976. Khorramshahr city has hot summers (up to 55°C) and cold winters (1°C). The annual rainfall is around 140 mm.

2.2. Study Design and Sample Collection. In this case-control study, a total of 105 cases including 36 patients with T1DM and 69 patients with T2DM were recruited from Vali Asr Hospital (affiliated to the Abadan Faculty of Medical Sciences) from December 2019 to March 2020. A total of 150 control subjects were also enrolled. Each case group had its own control group. In the diabetic groups, inclusion criteria were as follows: fasting plasma glucose greater than or equal to 7.0 mmol/L and/or 2-hour plasma glucose greater than or equal to 11.1 mmol/L [14]. Healthy individuals were defined as control group if they had no previous history of diagnosis of diabetes and had fasting and 2-hour glucose measures under the common thresholds for diabetes. The diabetic patients in both case groups with metabolic disorders and those receiving immunosuppressive drugs were excluded from the current research.

2.3. Questionnaire. Each participant completed a structured questionnaire which obtained the following demographic information: age, gender, residence, education level, contact with cat, source of drinking water, and consumption of raw or undercooked meat.

2.4. Serological Assay. All the patients and control subjects had 5 mL of venous blood drawn. The samples were then centrifuged at 1700 \( \times g \) for 5 minutes and kept at \(-20°C\) till tested. In order to detect anti-\( T. gondii \) IgG antibody titer in the sera, a commercially available (Torch-IgG, Trinity Biotech Company) enzyme-linked immunosorbent assay (ELISA) kit was used according to the manufacturer’s instructions.

2.5. Statistical Analysis. All data were imported into the Statistical Package for the Social Sciences (SPSS) software (version 21) (SPSS Inc., Chicago, IL, USA) for analysis. Chi-square and Fisher’s exact tests were used to compare the variables. The significance level was defined to be less than 0.05 (\( P < 0.05 \)).

3. Results

3.1. Seroepidemiology of \( T. gondii \) Infection in T1DM Patients. The seroprevalence of \( T. gondii \) infection in T1DM and control subjects was estimated to be 69.4% (25/36) and 34.0% (17/50), respectively, which showed a statistically significant difference (odds ratio (OR): 4.41; 95% confidence interval (CI): 1.75–11.06; \( P = 0.001 \)). Demographic characteristics of patients with T1DM and nondiabetic subjects, such as age group, gender, residence, education level, source of drinking water, and consumption of raw/undercooked meat, are presented in Table 1. T1DM patients in the age group of 21–30 years (80.0%) showed the highest seroprevalence. No significant difference was observed between females (82.35%) and males (57.89%) of T1DM patient group (\( P = 0.109 \)). T1DM patients who lived in rural areas (81.81%) had higher seroprevalence of \( T. gondii \) than those who were in urban regions (64.0%), but no statistically significant difference was observed (\( P = 0.254 \)). In addition, the seroprevalence of \( T. gondii \) infection in T1DM patients with different education levels was not significantly different (\( P = 0.261 \)). \( T. gondii \) seropositivity was not significantly different among T1DM patients with the history of contact with cats (\( P = 0.073 \)), source of drinking water (\( P = 0.571 \)), and consumption of raw/undercooked meat (\( P = 0.609 \)) (Table 1).

3.2. Seroepidemiology of \( T. gondii \) Infection in T2DM Patients. T2DM patients (72.5%) showed a higher seroprevalence of \( T. gondii \) infection than nondiabetic group (29.0%) (OR: 6.44; 95% CI: 3.25–12.74; \( P < 0.001 \)). T1DM patients in the age group of more than 60 years showed the highest rate of infection with \( T. gondii \) (73.91%). No significant difference was observed between males (67.64%) and females (77.14%) in T2DM patients (\( P = 0.377 \)). About 74.41% of T2DM patients living in urban areas were seropositive for \( T. gondii \), while in rural regions 69.23% were found to be IgG-positive (\( P = 0.64 \)). The seroprevalence of \( T. gondii \) infection was not significantly different in T2DM patients with different educational levels (\( P = 0.21 \)). \( T. gondii \) seropositivity was not significantly different among T1DM patients and source of drinking water (\( P = 0.292 \)) and consumption of raw/undercooked meat (\( P = 0.384 \)). Among risk factors, only contact with cats was significantly associated with IgG seropositivity in both T2DM patients (\( P < 0.001 \)) and control subjects (\( P = 0.045 \)) (Table 2).

4. Discussion

The possible association between toxoplasmosis and DM is still controversial, as there are several studies with conflicting results [11, 13, 15–17]. Since there is a lack of knowledge about the epidemiological status of \( T. gondii \) infection and its association with T1DM and T2DM in southwest Iran (Khuzestan province, Khorramshahr city), anti-\( T. gondii \) IgG antibody in diabetic patients compared to nondiabetic subjects was evaluated. Our findings showed higher seroprevalence of anti-\( T. gondii \) IgG antibody in
Table 1: Demographic characteristics and risk factors related to the seroprevalence of *T. gondii* infection in T1DM patients, Khorramshahr city.

| Characteristic                      | Type 1 DM (N = 36) | Controls (N = 50) | Type 1 DM versus controls |
|-------------------------------------|--------------------|-------------------|---------------------------|
|                                     | No. of tested IgG-positive % | No. of tested IgG-positive % | P value | P value |
| Age                                 |                    |                   |                         |
| 0–10                                | 5 3 60.00          | 8 2 25.00         | 0.847                   | 0.249   |
| 11–20                               | 9 6 66.66          | 12 4 33.33        | 0.142                   | 0.029   |
| 21–30                               | 10 8 80.00         | 15 5 33.33        | 0.029                   | 0.029   |
| 31–40                               | 12 8 66.66         | 15 6 40.00        | 0.029                   | 0.161   |
| Gender                              |                    |                   |                         |
| Female                              | 17 14 82.35        | 25 9 36.00        | 0.109                   | 0.003   |
| Male                                | 19 11 57.89        | 25 8 32.00        | 0.086                   |         |
| Residence                           |                    |                   |                         |
| Urban                               | 25 16 64.00        | 30 10 33.33       | 0.254                   | 0.023   |
| Rural                               | 11 9 81.81         | 20 7 35.00        | 0.023                   |         |
| Education level                     |                    |                   |                         |
| Diploma or lower                    | 27 20 74.07        | 35 12 34.28       | 0.261                   | 0.002   |
| University degree                   | 9 5 55.55          | 15 5 33.33        | 0.261                   | 0.026   |
| Contact with cat                    |                    |                   |                         |
| Yes                                 | 24 19 79.16        | 36 13 36.11       | 0.073                   | 0.002   |
| No                                  | 12 6 50.00         | 14 4 28.57        | 0.073                   | 0.237   |
| Source of drinking water            |                    |                   |                         |
| Unpurified water                    | 9 6 66.66          | 9 4 44.44         | 0.571                   | 0.319   |
| Purified water                      | 27 19 70.37        | 41 13 31.70       | 0.571                   | 0.002   |
| Consumption of raw/undercooked meat |                    |                   |                         |
| Yes                                 | 6 4 66.66          | 10 5 50.00        | 0.609                   | 0.451   |
| No                                  | 30 21 70.00        | 40 12 30.00       | 0.609                   | 0.001   |
| Total                               | 36 25 69.4         | 50 17 34.0        | 0.609                   | 0.001   |
T1DM and T2DM patients in comparison to nondiabetic individuals. Thus, the results of our study based on ELISA method supported the association between chronic toxoplasmosis and both types of DM.

T1DM is considered as an autoimmune disease, which is probably associated with genetic and environmental factors [10]. The association between infectious agents and T1DM has been approved [18, 19]. In this study, higher seroprevalence of \textit{T. gondii} infection in T1DM patients in comparison to nondiabetic individuals was observed (69.4% versus 34.0%). \textit{T. gondii} can infect all nucleated cells, including pancreatic β-cells. Pancreas can secrete insulin, which is crucial for controlling blood glucose level. Any deficiency in insulin production may cause the occurrence of T1DM [12, 16, 20]. In the other hand, the diabetic patients are considered as immunocompromised subjects and are more vulnerable to infection with \textit{T. gondii} than healthy individuals [21].

T2DM is a metabolic disease and, as a major global health concern, its incidence rate has increased during the recent decade throughout the globe [10, 22]. In the current study, 72.5% of T2DM patients and 29.0% of nondiabetic subjects were seropositive for \textit{T. gondii} IgG antibody, and the difference was statistically significant (P < 0.001). The same results were reported by Ozcelik et al. from Turkey [23]. In contrast with the results of our study, Molan et al. reported that 62.0% and 66.0% of the T2DM patients and nondiabetic subjects were seropositive for \textit{T. gondii} infection, respectively, but the difference was not statistically significant [17]. In a review paper with meta-analysis approach, Majidiani et al. reviewed seven articles to investigate the association between \textit{T. gondii} infection and DM from a global perspective. They concluded that latent toxoplasmosis accounts as a possible risk factor for T2DM (OR: 2.39; 95% CI: 1.20–4.75; P = 0.013), while no statistically significant association was observed between \textit{T. gondii} and T1DM (OR: 1.10; 95% CI: 0.13–9.57; P = 0.929) [13]. The discordance between studies could be explained due to the study area, the number of participants in the case and control groups, different type of sampling, environmental factors, lifestyle and habits of the people as well as different specificity and sensitivity of the laboratory techniques, variable cutoff values, or antibody titers for serological kits.

In the current research, the main risk factors of \textit{T. gondii} infection were assessed. A significant association between \textit{T. gondii} seroprevalence and contact with cats was found in both T2DM patients and nondiabetic subjects. In the previous studies among general population and patients undergoing hemodialysis, the same results were observed in southwest Iran [24, 25]. Since cats are considered as the only definitive hosts and are one of the major sources of \textit{T. gondii}, it seems that close contact with cats is considered as an important risk factor for acquiring the infection. The cats can release several millions of oocysts into the environment and public places through feces [1, 26]. In addition, the

### Table 2: Demographic characteristics and risk factors related to the seroprevalence of \textit{T. gondii} infection in T2DM patients, Khorramshahr city.

| Characteristic            | Type 2 DM (N = 69) | Controls (N = 100) | Type 2 DM versus controls |
|---------------------------|--------------------|--------------------|---------------------------|
|                          | No. of tested IgG-positive % | P value | No. of tested IgG-positive % | P value | P value |
| Age                       |                    |                    |                           |
| ≤40                       | 12                 | 8                  | 66.66                     | 0.884    | 25    | 7     | 28.00 | 0.03 |
| 41–60                     | 34                 | 25                 | 73.52                     | 0.001    | 50    | 14    | 28.00 | 0.93 |
| >60                       | 23                 | 17                 | 73.91                     | 0.004    | 25    | 8     | 32.00 | 0.001|
| Gender                    |                    |                    |                           |
| Female                    | 35                 | 27                 | 77.14                     | 0.377    | 50    | 13    | 26.00 | 0.509|
| Male                      | 34                 | 23                 | 67.64                     | 0.001    | 50    | 16    | 32.00 | 0.001|
| Residence                 |                    |                    |                           |
| Urban                     | 43                 | 32                 | 74.41                     | 0.64     | 60    | 19    | 31.66 | 0.472|
| Rural                     | 26                 | 18                 | 69.23                     | 0.001    | 40    | 10    | 25.00 | 0.001|
| Education level           |                    |                    |                           |
| Diploma or lower          | 51                 | 39                 | 76.47                     | 0.21     | 66    | 21    | 31.81 | 0.387|
| University degree         | 18                 | 11                 | 61.11                     | 0.001    | 34    | 8     | 23.52 | 0.007|
| Contact with cat          |                    |                    |                           |
| Yes                       | 52                 | 44                 | 84.51                     | <0.001   | 73    | 25    | 34.24 | 0.045|
| No                        | 17                 | 6                  | 35.29                     | 0.045    | 27    | 4     | 14.81 | 0.114|
| Source of drinking water  |                    |                    |                           |
| Unpurified water          | 7                  | 4                  | 57.14                     | 0.292    | 11    | 6     | 54.54 | 0.648|
| Purified water            | 62                 | 46                 | 74.19                     | 0.048    | 89    | 23    | 25.84 | <0.001|
| Consumption of raw/undercooked meat | 8                  | 5                  | 62.50                     | 0.384    | 14    | 7     | 50.00 | 0.062|
| No                        | 61                 | 45                 | 73.77                     | 0.001    | 86    | 22    | 25.58 | 0.454|
| Total                     | 69                 | 50                 | 72.5                      | 100      | 29    | 29.00 | <0.001|
sporulated oocysts have the ability to survive for a long time in the optimum conditions in the soil [5]. Based on a review paper, the prevalence of T. gondii oocysts was estimated to be 16% (95% CI: 10–26) in the soil of public places worldwide [5].

Choosing the appropriate inclusion and exclusion criteria, investigating the clinical and diagnostic history of all the participants, assessment of demographic information and the main risk factors of T. gondii infection through a structured questionnaire, and investigation of both T1DM and T2DM are strengths of the current study. Nonetheless, there are limitations that should be kept in mind: (1) this study was based on sampling from a small number of T1DM and T2DM patients in a limited area; (2) only serological assay by ELISA was performed on samples with no supporting data by molecular confirmation.

5. Conclusion

In conclusion, we found high rates of T. gondii seroprevalence in diabetic patients in southwest Iran. Although this study revealed a significant association between chronic T. gondii infection and two types of diabetes mellitus (T1DM and T2DM), there remain many questions regarding the exact mechanisms of T. gondii in the pathogenesis of DM. More studies are required to elucidate the exact association between T. gondii and DM.

Abbreviations

CI: Confidence interval
DM: Diabetes mellitus
ELISA: Enzyme-linked immunosorbent assay
IgG: Immunoglobulin G
OR: Odds ratio
T. gondii: Toxoplasma gondii
T1DM: Type 1 diabetes mellitus
T2DM: Type 2 diabetes mellitus

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethical Approval

This study received the approval from the Behbahan Faculty of Medical Sciences Ethical Committee (IR.BHN.REC.1399.008).

Consent

All subjects voluntarily agreed to be tested. A written informed consent was obtained from adult persons and parents or guardians of subjects below 18 years of age.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors’ Contributions

Shahrzad Soltani, Masoud Foroutan, and Sanaz Tavakoli conceived, designed, and drafted the manuscript; Mohamad Sabaghan, Mehdi Sagha Kahvaz, and Marzieh Pashmforosh contributed to data acquisition; Shahrzad Soltani and Masoud Foroutan contributed to statistical analysis; Masoud Foroutan critically revised the text. All authors read and approved the final version of the manuscript. The corresponding authors had access to the data in the study and had final responsibility for the decision to submit for publication.

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