ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine
www.aaem.pl

Toxocara infection in children with type 1 diabetes mellitus in Iran: a case-control seroprevalence study

Fatemeh Mirzaei[1,B,F], Fatemeh Aghamahdi[1,C,F], Mohammad Zibaei[2,3,A,C-D,F] ✉, Omid Safari[1,E], Shahab Noorian[1,E], Farzaneh Firoozeh[4,D,F], Malihe Farid[5,C]

[1] *Department of Pediatrics, School of Medicine, Imam Ali Hospital, Alborz University of Medical Sciences, Karaj, Iran*
[2] *Department of Parasitology and Mycology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran*
[3] *Center for Research of Endemic Parasites of Iran, Tehran University of Medical Sciences, Tehran, Iran*
[4] *Department of Microbiology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran*
[5] *Social Determinants of Health Research Center, Alborz University of Medical Sciences, Karaj, Iran*
A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

Mirzaei F, Aghamahdi F, Zibaei M, Safari O, Noorian S, Firoozeh F, Farid M. *Toxocara* infection in children with type 1 diabetes mellitus in Iran: a case-control seroprevalence study. Ann Agric Environ Med. doi:10.26444/aaem/208958

Abstract

Introduction and Objective. Parasitic diseases and subsequent immunity against helminth infections involves profound changes in both the innate and adaptive immune compartments, which can have a protective effect in inflammation and autoimmunity diseases such as type 1 diabetes mellitus. The aim of the study is to evaluate the prevalence of anti-*Toxocara* antibodies and associated risk factors among diabetic children in a case-control study.

Materials and Method. A total of 105 blood samples from diabetic children, and 101 non-diabetic controls attending the Comprehensive Imam Ali Children's Hospital, Alborz University of Medical Sciences in Karaj, Iran, were collected and tested by ELISA for anti-*Toxocara* antibodies. A questionnaire administered by direct interviews was used to collect socio-demographic information and data on associated risk factors.

Results. The total seroprevalence of anti-*Toxocara* antibodies was significantly higher in healthy control individuals (5.9%) than in diabetic children (1.9%) (COR: 0.341, 95% CI: 0.079–1.480; AOR: 0.168, 95% CI: 0.029–0.967). Furthermore, seropositivity for toxocariasis was higher in the uncontrolled diabetic group than in controlled diabetic children, this was statistically significant ($P = 0.046$). Female participants (62.5%) were more susceptible to *Toxocara* infection. Also, living in rural areas (62.5%), illiteracy (75.0%), and contact with non-domesticated animals and/or soil (75.5%) were found to be the highest risk factors for toxocariasis in the studied children.

Conclusion. The finding of the study supports the hypothesis of an association between parasitic helminths infections and diabetes mellitus, and that T1DM can be considered as a stimulus in regulating and modulating the immune system in toxocariasis. More accurate results could be obtained from a larger than necessary sample better representative of the population.

Key words

Type 1 diabetes mellitus, *Toxocara* infection, anti-*Toxocara* antibodies, ELISA

INTRODUCTION

Personal and environmental health is fundamental to society, as well as to individuals in need of special attention, as it has a major impact on the global burden of disease. The developing countries of Asia and Africa are at high risk of environmental factors which cause illness and death from helminthic infections, respiratory infections, and diarrhea [1].

Toxocariasis is a zoonotic parasitic disease which can infect a large number of mammals including humans, and is a major burden for public health worldwide. Dogs and cats are the definitive host of *Toxocara canis* and *Toxocara cati*, respectively [2]. These animals can become infected if they contact with dirt and ingest dirt containing *Toxocara* species eggs. Although rare, infection can also occur through eating undercooked or raw meat from infected paratenic hosts, such as mammalian and birds [3]. In paratenic hosts, such as humans, larvae can migrate through the tissues and cause ocular larva migrans, visceral larva migrans, neurotoxocariasis, and common, covert toxocariasis. A positive serological test result, such as ELISA, is indicative of a current or past infection in the affected population [4].

Globally, approximately 1.4 billion people (one-fifth) of the world's human population is exposed to *Toxocara*, and *Toxocara* seroprevalence varies considerably by country and region [5]. Humans are mainly affected by four major forms of toxocariasis, including visceral, ocular, nervous, and non-classic toxocariasis. Covert or non-classic toxocariasis is a mild, subclinical, febrile illness with various non-characteristic signs and symptoms, such as acute bronchitis, pneumonia with or without Löffler syndrome, which is seen in seropositive individuals [6].

Diabetes mellitus is a common chronic metabolic disease, and it is predicted that by 2030 more than 300 million people worldwide will be affected by the disease. Genetic and environmental factors can play an important role in determining

✉ Address for correspondence: Mohammad Zibaei Department of Parasitology, Alborz University of Medical Sciences, Square Talaghani, North Talaghani Blvd, 3149779453 Karaj, Iran
E-mail: zibaeim@sums.ac.ir

Received: 05.10.2024; accepted: 31.07.2025; first published: 29.04.2026

susceptibility to type 1 diabetes mellitus (T1DM) [7]. There are many environmental factors that play an important role in this increased incidence of type 1 diabetes (T1D), including exposure to bacterial and viral infections, dietary changes, or reduced exposure to specific infectious agents, such as certain helminthic infections that have historically played a role in T1DM. The results of studies have shown that some infectious agents such as *Helicobacter pylori*, hepatitis C virus and *Toxoplasma gondii* are associated with diabetes [7]. Immunity to helminths involves profound changes in both the innate and adaptive immune compartments, which can have a protective effect in inflammation and autoimmunity [8]. It has been determined in studies that antigens and molecules derived from worms play a role in the treatment of inflammatory and autoimmune diseases such as T1DM *in vitro* as well as *in vivo* conditions [9, 10]. A study has shown that molecules derived from tissue-dwelling helminth parasites, including *Trichinella*, especially the components of excretory-secretory products, have a role to play in the potential to improve immunopathology in animal models of various human inflammatory and autoimmune diseases [11].

In a previous study conducted on individuals with various helminth infections, positive toxocariasis was reported in a diabetic patient [12]. In Iran, a cross-sectional study has shown an association between *Toxocara* infection and diabetes. The results of the study showed that there is a significant relationship between toxocariasis and seropositivity in adult diabetic patients [13].

OBJECTIVE

Since data of previous studies on the association between *T. canis* infection and diabetes mellitus remain controversial, matched case-control studies were performed to determine whether *Toxocara* seropositivity is associated with T1DM, and explore the risk factors for *Toxocara* infection in diabetes children for the first time in Iran.

MATERIALS AND METHODS

Study population and design. An age, gender, and residence-matched cohort study was carried out between October 2023 – March 2024 on 105 T1DM children referred to the Imam Ali (A_s) Specialized Children's Comprehensive Hospital, Outpatient Clinic, School of Medicine, Alborz University of Medical Sciences in Karaj, Iran. All study participants were in different stages of diabetes and undergoing different cycles of drug therapy. Additionally, 101 non-diabetic subjects were recruited as a control group. T1DM childern were excluded from the control group by history taking and measuring their random blood sugar (Fig. 1). T1DM children were included based on their age (1 – 18 years-old), fasting plasma glucose (FPG) ≥ 126 mg/dL (7.0 mmol/L), 2-hour post-prandial plasma glucose (2-PP) > 200 mg/dL (11.1 mmol/L), as well as haemoglobin A1C (HbA1C) ≥ 7% (48 mmol/L).

A faecal examination was performed to rule out any other helminthic infections, such as *Ascaris lumbricoides*, *Hymenolepis nana* and/or Hookworms, which are common in children and young adults.

Independent variables data were collected directly from the participants – the children themselves or their relatives. Demographic and lifestyle characteristics were obtained through a survey questionnaire which included such information as gender, age, educational levels, residency, and potential risk factors for *Toxocara* infection (exposure to domestic animals and/or soil, and pet keeping).

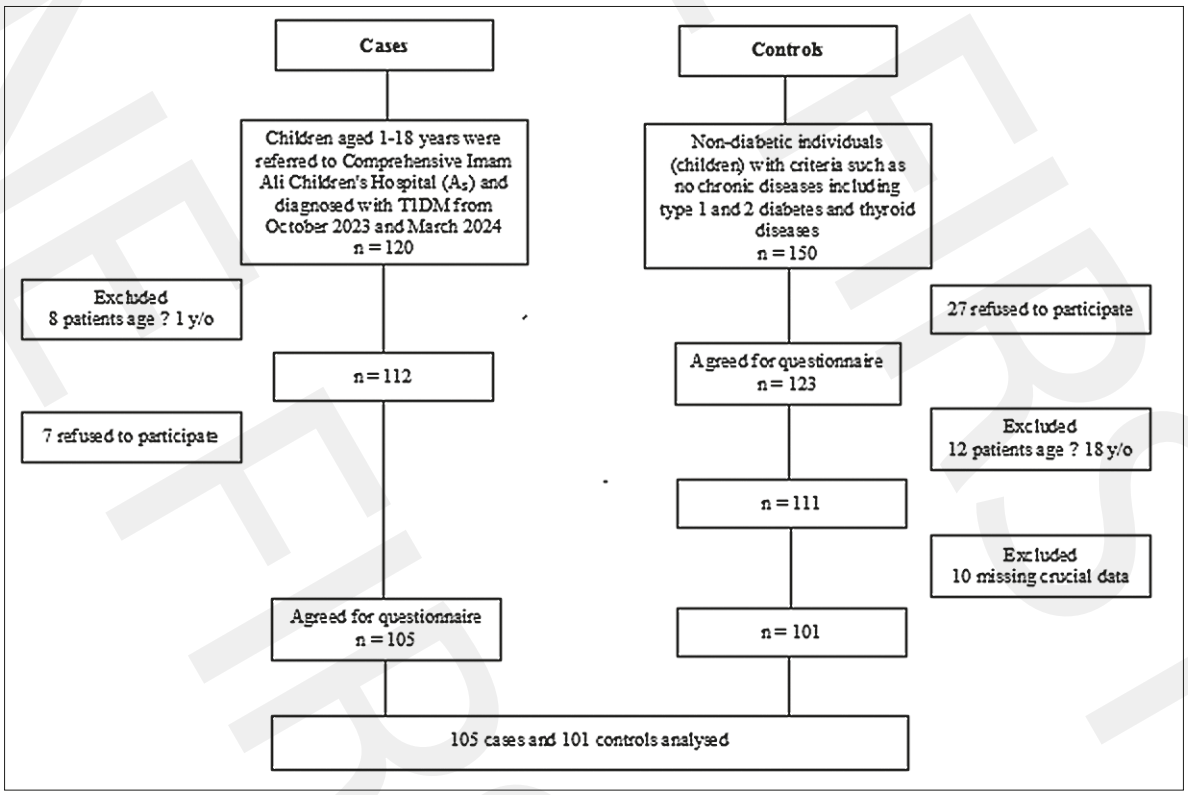

Figure 1. Flowchart of participant recruitment in the case-control study

Laboratory analyses. From each subject in both groups, 5 milliliters of venous blood were taken under sterile conditions, and the serum of the blood samples was separated by centrifugation at 2,500 rpm and stored in a sufficient amount at -20 °C for serological assay.

Anti-*Toxocara* antibodies were detected using an enzyme-linked immunosorbent assay (ELISA) kit (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany), which can achieve high diagnostic sensitivity and specificity (more than 95%), and the reference method considered for serological diagnosis. Briefly, sera diluted 1:100 with sample diluent (100 µL/well) were used in duplicate and incubated at 4 °C for 1 h. After washing 3 times in washing solution, the plates were incubated with 100 µL/well of *Toxocara* protein A horseradish peroxidase conjugate at room temperature for 30 min. The microplates were then incubated with 100 µL of tetramethylbenzidine (TMB) substrate for 15 min at 37 °C. The reaction (100 µL/well) was stopped with stop solution for 15 min at room temperature. Optical density (OD) was determined at 450/620 nm for each well in a microplate reader (STATFAX-2100-OHAHIO-USA). According to the manufacturer, the assay is validated if the absorbance (OD) of blank is less than 0.100; the mean absorbance of the negative control is less than 0.200, the absorbance of the cut-off is between 0.150 and 1.30, and the absorbance of the negative control is greater than the cut-off point.

Statistical analysis. Results were analyzed with SPSS 26.0 software package (IBM Corp., Armonk, NY, USA). For univariate analysis, Chi-square (x^2) test was used to compare the categorical variables. The Mantel-Haenszel test was used to probe any differences between the patient and control groups. Multivariate regression models were used to adjust for potential confounders. Odds ratios (ORs) and the corresponding 95% confidence interval (CI) were calculated in order to identify the independent risk factors for *Toxocara* infection. Results with a p value < 0.05 were considered as statistically significant.

Ethical considerations. The study protocol was ethically approved by the Ethics Clearance Committee of the Alborz University of Medical Sciences (ECCABZUMS) (IR.ABZUMS.REC.1401.225). The participants were included after providing written informed consent. All personal information and identifiers of the study participants were kept conditionally, and those who were positive for any intestinal helminthes were treated with antihelminthic drugs. All methods were carried out in accordance with relevant guidelines and regulations of ECCABZUMS.

RESULTS

The overall seroprevalence of *Toxocara* species infection in the diabetic children and the control group was 1.9% (2/105) and 5.9% (6/101), respectively (AOR: 0.168, 95% CI: 0.029–0.967, $P = 0.046$) (Tab. 1).

Details of the socio-demographic characteristics of the participants, including age, gender, educational level, area of residence, pet keeping, contact with non-domesticated animal and/or soil, as well as laboratory data of FPG and HbA1c levels, are shown in Table 2.

The gender, age group, level of education, pet keeping, and place of residency showed no statistically significant difference between the seropositive and seronegative diabetic children ($P > 0.05$). Children who had contact with non-domesticated animals and/or soil (75.0%) had a higher significant *Toxocara* seroprevalence compared to with those who had no contact (25.0%) (AOR: 8.468, 95% CI: 1.240–57.849; $P = 0.035$). Children with FPG levels above 130 mg/dL (87.5%) had a higher seroprevalence than those with lower serum levels (12.5%), which was statistically significant (AOR: 8.72, 95% CI: 7.15–8.13; $P = 0.028$). HbA1c in the studied children was at a significant level (AOR: 11.01, CI 95%: 5.21–19.34; $P = 0.023$).

DISCUSSION

The association between toxocariasis and diabetes mellitus remains controversial, with some studies reporting conflicting results [12, 13]. The present study was performed with the aim of determining whether *Toxocara* infection is associated with type 1 diabetes mellitus in children in Iran. The results showed that T1DM patients had lower frequencies of antibodies against *Toxocara* compared to control subjects. The results obtained, based on serological methods, therefore confirmed the hypothetical association between T1DM and *Toxocara* infection.

Type 1 diabetes mellitus is an autoimmune disease with complex interactions between genetic and environmental factors. The infectious agents were found to be associated with T1DM [8]. Since 1990, a number of studies have been conducted on the correlation between parasitic diseases and diabetes [14]. The precise pathogenetic role of some helminth infections, such as toxocariasis in type 1 diabetes, is still a matter of debate, and the process underlying this association is unclear. Epidemiological and serological studies, as well as some eradicating trials, produced conflicting results [15].

In the current study, the overall seroprevalence of anti-*Toxocara* antibodies in the control subjects (5.9%) was significantly higher than in the diabetic patients (1.9%; $P = 0.046$). This is consistent with Zaccone et al. [16], but in contradiction to Raisi et al. [13] who reported that *Toxocara* infection was more common in adults with diabetes mellitus.

Parasitic infections, especially helminthic diseases, adapt to their host during prolonged infections, usually resulting in chronic disease with reduced mortality. Therefore, despite the pathogenicity of parasitic worms, they protect their host against some inflammatory diseases by modulating the immune mechanisms [8]. This has been investigated in many experimental animal models of the inflammatory disease, including T1DM, colitis, allergy, asthma, and gastric atrophy [9]. The helminthic infections elicit modulated Th2

Table 1. Seroprevalence of antibodies to *Toxocara* in children with T1DM[a] and non-T1DM[b] with logistic regression analyses

Children	Total, N (%)	Diabetic patients, N (%)	Non-diabetic (Controls), N (%)	COR (CI 95%)†	P- value	AOR (CI 95%)††	P- value
Seropositive	8 (3.9)	2 (1.9)	6 (5.9)	0.341 (0.079–1.480)	0.151	0.168 (0.029–0.967)	0.046*
Seronegative	198 (96.1)	103 (98.1)	95 (94.1)				
Total	206 (100.0)	105 (100.0)	101 (100.0)				

[a]Type 1 diabetes mellitus; [b]non-type 1 diabetes mellitus; †COR – c rude odds ratio with 95% confidence interval; ††AOR – adjusted odds ratio with 95% confidence interval; *P values ≤ 0.05 interpreted as statistically significant

Table 2. Factors related to toxocariasis in children with diabetes mellitus type 1 and healthy individuals - multivariate logistic regression

Characteristics	Statistical analysis		Total	COR (CI 95%)	P- value	AOR (CI 95%)	P- value
	Positive cases (%)	Negative cases (%)					
Age group (years)							
< 9 (1-8)	2 (25.0)	103 (52.0)	105 (51.0)	0.989 (0.841-1.162)	0.891	1.004 (0.832-1.21)	.0.966
≥ 9 (9-18)	6 (75.0)	95 (47.0)	101 (49.0)				
Gender							
Female	5 (62.5)	112 (56.6)	117 (56.8)	1.201 (0.277-5.198)	0.807	1.609 (0.317-8.165)	0.568
Male	3 (37.5)	86 (43.4)	89 (43.2)				
Residency							
Urban	3 (37.5)	126 (63.6)	129 (62.6)	0.509 (0.123-2.116)	0.353	1.159 (0.153-8.979)	0..887
Rural	5 (62.5)	72 (36.4)	77 (37.4)				
Education							
Illiterate	6 (75.0)	98 (49.5)	104 (50.5)	1.238 (0.580-2.643)	0.581	1.092 (.0363-3.288)	0.876
Primary School	1 (12.50)	65 (32.8)	66 (32.0)				
High School	1 (12.50)	35 (17.7)	36 (17.5)				
Pet keeping							
Yes	2 (25.0)	54 (27.3)	56 (27.2)	1.190 (0.230-6.159)	0.835	2.190 (0.318-15.094)	0.426
No	6 (75.0)	144 (72.7)	150 (72.8)				
Contact with non-domesticated animal and/or soil							
Yes	6 (75.0)	81 (40.9)	87 (42.2)	4.742 (0.928-24.241)	0.042*	8.468 (1.240-57.849)	0.035*
No	2 (25.0)	117 (59.1)	119 (57.8)				
Fasting plasma glucose (FPG)							
≥ 130 mg/dL	1 (12.5)	185 (93.4)	186 (90.3)	8.68 (7.17-9.24)	0.039*	8.72 (7.15-8.13)	0.028*
< 130 mg/dL	7 (87.5)	13 (6.6)	20 (9.7)				
Hemoglobin A1C (HbA1C)							
≥ 7% (48 mmol/L)	0 (0.0)	193 (97.5)	193 (93.7)	11.43 (5.74-22.76)	0.022*	11.01 (5.21-19.34)	0.023*
< 7% (48 mmol/L)	8 (100.0)	5 (2.5)	13 (6.3)				
Total	8 (100.0)	198 (100.0)	206 (100.0)				

*Significant association by the χ^2 test

responses in their hosts, leading to an immune response with a well-controlled inflammatory component, including inhibition of pro-inflammatory cytokines and induction of hyporesponsive state by Interleukin-10 (IL-10), and produces Treg-cells [17]. T-regulators populations have been shown to play a role in suppressing host immunity and regulating parasite fertility [8, 17]. Saunders et al. [15] showed evidence of strong association between T1DM and serological markers of infection sustained by intestinal parasites. In 2015, Elnadi et al. [19] reported a higher prevalence of intestinal parasites in patients with type 1 and type 2 diabetes. This finding has been confirmed by some studies [20].

In a case-control study by Akinbo et al. [21], 150 diabetic patients (diabetes type 1 and type 2) and 30 non-diabetic individuals were included, and their stool samples examined for intestinal parasites. The results showed that the prevalence of intestinal parasites in patients with type 1 and type 2 diabetes was higher than in healthy people, but no significant difference in the prevalence of intestinal parasites was observed in those with 2 types of diabetes. In the current study, the number of children with *Toxocara* in the healthy control group was higher than in the children with T1DM. This difference in the results could have been due to the intestinal parasites and visceral parasites being examined by 2 different methods, as well as by the small number of samples in the control group of the previous study. In a study by Mohtashmipour et al. [22], the prevalence of intestinal parasites in diabetic people (age group 4–73 years) was higher than in healthy people (26.3% and 8.6%, respectively). The difference in results may be due to the fact that the study was conducted in different age groups, different parasites were examined, and the presence of both groups of people with diabetes type 1 and diabetes type 2.

The risk of exposure to *Toxocara* infection is higher in the first 2 decade of the life for various reasons, including, geophagia behaviour in children, close contact with a definitive host (mainly dogs and cats), and playing with soil. *Toxocara* infection is also acquired by ingesting larval eggs found in public places, and contact with non-domestic (stray) dogs or cats [23]. In the current study it was found that *Toxocara* infection was most prevalent among individuals who had contact with non-domestic dogs and cats and/or soil; multivariate analysis showed that failure to wash hands after contact with pets was statistically a risk factor associated with toxocariasis. This finding is consistent with other published data showing a strong association between contact with animals, especially non-domestic animals (stray), and the development of toxocariasis [24].

The findings of the present study confirmed that the probability of infection of the studied females (62.5%) was higher than males (37.5%) with *Toxocara* species; similar results were also observed in previous studies [13]. It is hypothesized that the reasons for such a high prevalence can be attributed to different behavioural attitudes and contact with pets and non-domestic animals. However, the gender of patients was not significantly associated with *Toxocara* seroprevalence in either the univariate or multivariate analysis in both groups in the current study.

The participants in the current study included different age groups, from infants to young adults, with ages ranging

from 1–18 years. As suggested by Akinbo et al. [21], *Toxocara* infection is more common in childhood (between the ages of 5–15 years) because hygiene habits are not yet fully developed.

The seroprevalence of anti-*Toxocara* antibodies was higher in children living in rural areas (62.6%) than in those living in urban areas. The lifestyle of residents of rural areas, poor sanitary conditions, and contact with domestic animals could well explain this significant seroprevalence. It can also be explained by the fact that *Toxocara* eggs excreted by dogs and cats contaminate the soil, and the eggs can remain infectious for several months or even years.

In the current study, there was a non-significant association between the education level of the diabetic patients and the seroprevalence of *Toxocara* ($P = 0.876$). Similarly, Zibaei et al. [22] reported that the prevalence of anti-*Toxocara* antibodies was not statistically significant in relation to level of education.

Regarding of FPG and HbA1C levels, the seropositivity for toxocariasis was higher in the uncontrolled diabetic group (87.5%, FPG < 126; 100%, HbAIc < 7) than in the controlled diabetic children. This was statistically significant and consistent with a previous study (2022) by Elkholy et al. [25]. This finding may be due to the glycation of immunoglobulin molecules that occurs in diabetic individuals, which affect the biological function of antibodies, and thereby their vulnerability to infections.

Limitations of the study. It is worth mentioning some limitations which constrained the findings obtained in the study, including the absence of a standard, easy-to-use diagnostic tool in case of *Toxocara* infection, very limited studies regarding the relationship between helminthic parasitic infections, especially toxocariasis and T1DM, and lack of investigations considering different aspects of human *Toxocara*-induced complications, such as visceral and ocular larva migrans syndromes, neurotoxocariasis, and covert toxocariasis.

CONCLUSIONS

The study provides serological evidence of an association between *Toxocara* infection and type 1 diabetes mellitus in children. Moreover, fasting plasma glucose and haemoglobin A1C levels, and contact with non-domesticated animals (stray dogs and cats) or soil, were risk factors for *Toxocara* seropositivity, obtained by using multivariate regression, which may assist in guiding future research policies. Further studies should be conducted to elucidate the role of *Toxocara* in diabetes mellitus.

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