



Toxocara Infection in Asthmatic Children: A Case-Control Study in Karaj District, Iran

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Abstract

Background: It has been proposed that the migration of *Toxocara canis* larvae through the lungs is a contributing factor in the development of asthma and association of asthma and toxocariasis has been reported. The seroprevalence of toxocariasis varies widely among children living in different countries and even in different regions of a single country.

Objectives: The aim of this study is to compare seroprevalence of anti-Toxocara antibodies in asthmatic and healthy children in Karaj, Iran.

Methods: A serological study was conducted in children aged 1 to 15 years old for detection of antibodies to *Toxocara canis* in two groups (92 asthmatic children and 100 non-asthmatic children as control group), using commercial ELISA method for determination of IgG antibodies against Toxocara.

Results: Only one patient (1.09%) had positive levels of anti-Toxocara antibodies in the case group, while no one had them in the control group. The results showed that there was no significant correlation between IgG antibodies against *Toxocara canis* and asthma. More than 90% of children in both case and control groups were residents of urban areas.

Conclusions: We did not find any association between Toxocara infection and childhood asthma, suggesting that it may not be a common risk factor for childhood asthma in our urban area.

Keywords: Asthma, Child, Toxocara, Iran

1. Background

Asthma is the most common chronic disease in pediatric population and it is a worldwide health problem (1). The prevalence of childhood asthma varies considerably among countries and has increased over the last two decades in many countries. The reasons of these differences are not well understood and require further study to evaluate risk factors for asthma in each region (1, 2).

According to some epidemiologic and two meta-analysis studies, a positive association exists between asthma and *Toxocara* species' seropositivity, and toxocariasis can be a risk factor for childhood asthma in some regions (3, 4). *Toxocara canis* (*T. canis*) is an important zoonotic parasite and causes human toxocariasis worldwide. Humans can be infected with this parasite through accidental ingestion of eggs. In the small intestine, the eggs hatch to release larvae that penetrate through the in-

testinal wall and then migrate through the portal circulation to the lungs, and other organs, where they can survive for a long time (5). It has been proposed that the migration of *T. canis* larvae to the lungs and induction of persistent inflammatory reaction, eosinophilia, IgE production, airway hyper responsiveness, and production of T-helper cell type 2 cytokines are underlying factors in the development of asthma (6, 7). Although toxocariasis is a global infectious disease, the prevalence of Toxocara seropositivity varies from country to country and region to region (8).

The reported seroprevalence of anti-Toxocara antibodies in children of Iran varies widely across studies, from 1.39% in Isfahan to 25.6% in Shiraz (9-11). However, to the best of our knowledge, there is no study on seroprevalence of human toxocariasis among the children of Karaj. In addition, the prevalence of Toxocara seropositivity in asthmatic subjects has rarely been reported in Iran.

2. Objectives

The aim of this study is to compare seroprevalence of anti-Toxocara antibodies in asthmatic and healthy children in Karaj, Iran.

3. Methods

3.1. Study Area and Population

The study population consisted of asthmatic children aged 2 - 15 years who were residents of Karaj city or its rural area. By convenience sampling methods, 92 patients referring to the allergy and immunology clinic at Shahid Bahonar Hospital between February 2016 and March 2017 were selected as the case group.

Karaj is a city in the western part of the Tehran province and one of the metropolises of Iran. The patients were selected from the allergy and immunology clinic. The inclusion criteria for this study were diagnosis of asthma and age between 2 to 15 years old. The diagnosis of asthma was made if all the following criteria were met: (1) Recurrent episodes of wheezing or coughing or breathing difficulty that occurs in the absence of a respiratory infection, (2) symptoms vary over time and vary in intensity, (3) symptoms improve with salbutamol or in children who can do spirometry there is increase in FEV1 of > 12% predicted after using 200 - 400 mcg salbutamol. The presence of aggravating factors such as, physical exercise, laughing, crying or cold weather as well as presence of personal or family history of atopy were confirmatory criteria for diagnosis.

The control group was selected from general pediatric clinics from the same hospital out of patients who referred to physicians for checkup or diseases other than respiratory problems. The exclusion criteria for this group included any evidence for present asthma or chronic cough.

All of the children and their parents answered a questionnaire containing epidemiological data such as age, sex, presence of a pet animal (dog or cat) in the house or contact with stray dogs, habits of geophagia, and atopic status.

Atopic status in this study was defined as self-report of ever having physician-diagnosed atopic dermatitis or hay fever in the child or presence of asthma, atopic eczema or allergic rhinitis in his/her parents.

In addition, the degree of asthma control was assessed through specialist questionnaire, prepared based on the GINA (Global Initiative for Asthma) guideline (2015) (12).

3.2. Serum Samples

Blood samples were collected from 192 children of whom 92 subjects had asthma, while the rest did not have this condition. The samples were tested for total IgE, CBC, differential and the presence of anti-Toxocara antibodies, using enzyme-linked immunosorbent assay (ELISA). Anti-Toxocara antibodies were detected by commercial ELISA kits (NovaLisa Novatec, Immundiagnostica GmbH, Germany product number: TOCG0450, 96 determinations) according to the manufacture's instruction. Based on those instructions, the result was considered positive when a value higher than 11.0 U/mL was recorded.

We double-checked anti-Toxocara antibodies for all of the borderline cases and patients who had hyper eosinophilia in both case and control groups.

3.3. Ethical Consideration

The study protocol was approved by the Ethics Committee of the Alborz University Medical Sciences, Iran (Abzums.rec.1394.73), and patients or their parents signed informed consent forms before starting the study.

3.4. Statistical Analysis

For statistical analyses, we used SPSS software version 15.0. Quantitative and qualitative parameters were evaluated by independent sample test and chi-square, with $P < 0.05$ considered statistically significant.

4. Results

A total of 192 children participated in the study: 100 controls without asthma and 92 case patients with asthma. The mean age of the case patients was 7.3 ± 2.8 years and that of the control group was 7.09 ± 3.8 years old. The two groups did not differ significantly in terms of distribution of sex, age, area of residency, geophagia habit and contact with stray dogs or pet ownership. The results also indicated that paternal education level was significantly lower in the case group than in the control group ($P = 0.02$). [Table 1](#) provides the demographic characteristics of the case patients and controls.

Children with asthma were significantly more likely to be atopic than the control group ($P < 0.001$). Further, 34.8% of asthmatic children had well-controlled asthma and both of partly controlled and uncontrolled asthma groups constituted 32.6% of the patients.

Only one patient (1.09%) had positive levels of anti-Toxocara antibodies in the case group, while no one had the condition in the control group. The patient with positive anti-Toxocara *canis* antibodies was a 15-year-old boy

Table 1. Demographic Characteristics of the Case Patients and Controls^a

Variables	Asthmatic Group	Control Group	P Value
Sex			0.258
Male	58 (63)	55 (55)	
Female	34 (37)	45 (45)	
Residency			0.17
Urban	84 (91.3)	96 (96)	
Rural	8 (8.7)	4 (4)	
Number of family members			0.61
3	33 (35.9)	41 (41)	
3 - 5	53 (57.6)	55 (55)	
> 5	6 (6.5)	4 (4)	
Father education			0.02
Less than high school diploma	34 (37)	17 (17)	
High school diploma or more	58 (63)	83 (83)	
Mother education			0.069
Less than high school diploma	30 (32.6)	21 (21)	
High school diploma or more	62 (67.4)	79 (79)	
Atopy history			< 0.001
Yes	64 (69.6)	25 (25)	
No	28 (30.4)	75 (75)	
Ownership or contact with dogs			0.11
Yes	6 (6.5)	2 (2)	
No	86 (93.5)	98 (98)	
Geophagia			0.34
Yes	5 (5.4)	9 (9)	
No	87 (94.6)	91 (91)	
Use of public parks			0.24
	26 (28.3)	21 (21)	

^a Values are expressed as No. (%).

who did not have eosinophilia or high IgE and he was resident of an urban area, but he had a history of contact with stray dogs. Three-time stool examination was done for him to rule out other intestinal helminthic infections including *Ascaris lumbricoides*, with the result being negative.

The results suggested no significant correlation between IgG antibodies against *T. canis* and any variable including sex, age, area of residency, geophagia, pet ownership and asthma.

IgE in the case group was significantly higher than in control group ($P < 0.001$). Also, eosinophil count was higher in the case group, though it was not statistically significant (Table 2).

5. Discussion

There are common characteristics for asthma and toxocarasis, such as persistent airway hyper-responsiveness and inflammation, eosinophil accumulation in lungs and induction of production of IgE (13, 14).

Our results failed to demonstrate an association between *Toxocara* infection and childhood asthma as with another study in Iran conducted by Mosayebi et al. (15) in the city of Arak, reporting two positive sera in 110 children with asthma (1.8%) and no one in 70 children in the control group. The limitation of their study was that the criteria for diagnosing asthma were unknown and they excluded patients with allergic or genetic asthma from the study whilst it has reported that in schoolchildren approximately 60% -

Table 2. Comparison of Case and Control Groups According to Lab Data^a

	Case Group	Control Group	P Value
IgE, median (IQR)	55 (180.25)	14.5 (36.75)	0.000
AEC, median (IQR)	190 (236)	173 (156)	0.155
Eosinophilia, No. (%)			0.19
Yes	15 (16.3)	10 (10)	
No	77 (83.7)	90 (90)	
Anti ToxocaraAb, %			0.29
Positive	1.09	0	
Negative	98.01	100	

Abbreviation: AEC, absolute eosinophil count.

^a Eosinophilia: AEC > 400.

90% of the asthmatic children are found to be allergic (16, 17).

A meta-analysis study including seven studies on children and three studies on adults involving a total of 1530 participants (723 cases of asthma and 807 controls) revealed a significant association between Toxocara seropositivity and asthma (OR: 3.36, 95% CI: 1.76 - 6.42) (3). However, another study that was performed in Brazil with inclusion criteria similar to those used in our study did not find this association (18).

Nevertheless, another recent meta-analysis published in 2018 with 17 studies (a total of 5469 participants, 872 asthmatics, and 4597 non-asthmatics children) indicated again an increased risk for asthma in children with Toxocara infection seropositivity (OR: 1.91, 95% CI: 1.47 - 2.47) (4).

Our different findings could be due to the following reasons. First, the lower prevalence of exposure in our area might attenuate the association. Secondly, the results of meta-analysis studies might have bias because of the heterogeneity among studies due to difference in definition of asthma, methods of case and control selection, geographic and environmental conditions in each region and methods employed for measuring anti-Toxocara antibodies. Furthermore, there is a concern that studies with positive, significant results (positive studies) are more likely to be published and as a result be introduced in meta-analysis studies, than studies with non-significant or negative results (negative studies) (19).

In Iran, the prevalence of *T. canis* varies widely. Sharif et al. (20) in 2010 suggest that the overall seroprevalence for Toxocara antibodies is 24.5% in schoolchildren in Sari, northern Iran. In 2015, Hosseini-Safa et al. (9) reported 1.39% positive seroprevalence for toxocariasis in children between 5 to 15 years old in Isfahan. Momeni et al. (21) had a study on 2-to-20-year-old children in Urmia, north-

west of Iran. They found 3% positive samples for this parasite. Their study indicated risk factors such as mother's educational level, keeping dogs or cats as pets, and history of coughing for Toxocara infection. In Zanjan, seroprevalence of anti-Toxocara antibodies was reported to be 1.6% in children from urban areas and 4.4% in rural areas (22).

We found the prevalence of anti-Toxocara antibodies in asthmatic children who lived in Karaj to be 1.09%, which is the minimum prevalence reported in Iran. We expected higher prevalence rates as Karaj is a large immigrant city in the neighborhood of Tehran and many of its population consist of workers with a relatively low socioeconomic status. However, surprisingly the prevalence in both case and control groups was very low.

There are some reasons for the low frequency of this infection among children of our study. First, more than 90% of children in both the case and control groups were residents of urban areas, where there are various reports suggesting that living in rural areas is an important risk factor for toxocariasis. In a survey, Dogan et al. (23) investigated seroepidemiological rate for Toxocara infection in the northwest of Turkey and found that 16.7% children from rural areas had a positive level of anti-Toxocara antibodies, while it was only 0.71% in the urban areas. Living in rural areas and keeping a dog in the house were significant risk factors for this infection in this study. Similarly in Iran, Mahmoudvand et al. (24) found the seroprevalence of *T. canis* infection to be 2.1% in children of 2 to 15 years old referring to health centers of Lorestan province in urban regions and 11.9% in rural regions. Living in rural regions (P = 0.018) and contact with dogs were significant risk factors for *T. canis* seropositivity in this province. Children living in rural areas, especially in our country, are more likely to be exposed to other risk factors such as contact with dogs, low parental educational level, poor personal hygiene and use of non-piped water (25, 26).

Having a pet is uncommon in Karaj because of religious inhibition and small apartments in urban areas. In our study, only six cases among the asthmatic children and two in the control group had history of contact with stray dogs or kept dogs in their houses, with five of them living in rural areas.

The main limitation of our study lies in the relatively small size of groups studied. However, most other published studies investigating this association have also had around 100 cases or less (3, 4). Also, it seems that more studies with larger samples in high prevalence regions and rural areas are required to investigate the impact of Toxocara infection in development of asthma.

The principal strengths of this study include, first,

good case-control matching especially in demographic characteristics. Secondly, asthma was diagnosed based on GINA criteria, which is an international guideline.

5.1. Conclusions

We did not find any association between *Toxocara* infection and childhood asthma, and *Toxocara* is not a prevalent risk factor for childhood asthma in urban area of Karaj. Nevertheless, it should be considered among subjects with risk factors such as living in rural areas or owning pets.

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Footnotes

Authors' Contribution: Homa Sadri: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, administrative, and technical and material support. Mohammad Javad Gharavi: study concept and design, acquisition of data, administrative, technical and material support, and study supervision. Reza Arjmand: study concept and design, acquisition of data. Mohammad Zibaei: study concept and design, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content. Nasrin Elahimehr: acquisition of data. Yasaman Shaker: drafting of the manuscript and statistical analysis.

Conflict of Interests: It is not declared by the authors.

Ethical Approval: This research has already been conducted as a research project at Alborz University of Medical Science and studied at the Ethics Committee of this university and received the code of Ethics No. 1394,73.

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