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Original Article

Seroprevalence of *Toxocara* Infection among Asthmatic Children in Shiraz City, Southern Iran

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Abstract

Background: Human toxocariasis is caused by *Toxocara canis* and *T. cati*, the nematodes in the intestine of dogs and cats, respectively. Since the association between asthma and toxocariasis is controversial, the aim of the present study was to investigate the seroprevalence of *Toxocara* infection among asthmatic children in comparison with healthy children.

***Methods*:** This case-control study was conducted on 92 asthmatic and 91 healthy children aged 1-16 years old in Shiraz City, Southern Iran in 2019-2020. The serum samples were tested for IgG anti-*Toxocara* antibodies by ELISA method using the *T. canis* larval excretory-secretory (E/S) antigens. The collected data were analyzed using SPSS software.

***Results*:** The seroprevalence of toxocariasis in asthmatic patients was higher than the healthy children with no significant difference in *Toxocara* seropositivity between two groups (9.8% vs 8.8%, $P = 0.817$). The association between *Toxocara* infection and variables such as gender and age were not statistically significant.

***Conclusion*:** There was no significant association between toxocariasis and childhood asthma. Further study on different regions such as urban and rural areas with a large sample size and using questionnaire for considering risk factors of asthma and toxocariasis is recommended.

Introduction

Human toxocariasis is a zoonotic disease caused by *Toxocara canis* and *T. cati*, the nematodes in the intestine of dogs and cats, respectively (1). Humans can be infected by the ingestion of embryonated *Toxocara* eggs in contaminated soil, raw vegetables or the consumption of encapsulated *Toxocara* larvae in the undercooked or raw meat of paratenic hosts accidentally (2). After the ingestion of *Toxocara* eggs or larvae, the second stage larvae cannot develop into the adult nematode in the human and migrate to different organs. Depending on the location of the larvae in the body, there are several forms of human toxocariasis, namely, visceral larva migrans (VLM), ocular larva migrans (OLM), neurotoxocariasis and covert toxocariasis (1). Human toxocariasis has a worldwide distribution and its prevalence rate has been estimated from 1.07% to 33.67% from different parts of Iran (3).

Asthma is a chronic respiratory disease that causes sporadic breathing difficulties. It does not have a specific cause. It is possibly caused by a range of risk factors including genetic factors, host factors and environmental factors (4). Some parasites such as *Toxocara* have been considered as the etiologic agents of asthma (5). In many cases of human toxocariasis, pulmonary symptoms such as coughing, wheezing and dyspnea are reported (1). The correlation between asthma and toxocariasis has been hypothesized. Some studies have reported a significant association between asthma and toxocariasis, and *Toxocara* as a risk factor for asthma has been considered (6-13). In contrast, some studies revealed no association between toxocariasis and asthma (14-21).

Toxocariasis is more common in children because of their high contact with soil when playing in public parks and playgrounds contaminated with *Toxocara* eggs (1). Since the association between asthma and toxocariasis is controversial, the current study aimed to investigate the seroprevalence of *Toxocara* infection among asthmatic children in comparison with healthy children.

Materials and Methods

Study area and Participants

This case-control study was conducted on asthmatic and healthy children aged 1-16 years old who referred to health centers of Shiraz University of medical sciences, Shiraz City, southern Iran, from October 2019 to February 2020. The study population consisted of 92 asthmatic children and 91 healthy children. The asthmatic children were diagnosed by a pulmonologist. The inclusion criteria for the asthmatic patients were the diagnosis of asthma, age between 1-16 years old and the consent of parents. The patients with allergic and genetic asthma were excluded from the study. The control group included children who referred to health centers for check-up and had no respiratory diseases. A structured questionnaire was used to obtain the data such as gender and age of participants.

The design of the study, including ethical aspects, was approved by the ethics committee of the Shiraz University of Medical Sciences, Iran (code: IR.SUMS.MED.REC.1397.184), and the informed consent forms was signed by the children parents.

ELISA assay

About a 3 mL, venous blood sample was collected from all participants in both groups. The serum was separated and tested for IgG anti-*Toxocara* antibodies by ELISA method using the *T. canis* larval excretory-secretory (E/S) antigens prepared using the method previously described (22). For the ELISA method, flat-bottom 96-well microplate (corning, USA) was coated with 5 µg/mL of *Toxocara* E/S antigen overnight at 4°C. Plate was washed five times with washing buffer (PBST,

0.05% Tween 20 in PBS). Blocking was performed with 5% skimmed milk in PBST for 1 hour at room temperature.

The plate was washed again and 100 µL of serum sample (1/100 dilution in PBST) was added to each well and incubated for 1 hour. After washing, 100 µL of horseradish peroxidase-conjugated goat anti-human IgG (Sigma, USA, 1/4000 dilution in PBST) was added to each well in the plate and incubated in 37 °C for 1 hour. After washing, the plate was incubated with 100 µL/well of the substrate (0.4 mg/mL OPD, 0.3% H2O2 in 0.1 M citrate buffer, pH=5.6) for 20 minutes to visualize the reaction. The optical density (OD) values were measured at a wavelength of 490 nm, using an ELISA plate reader (ELx800, Bio-Tek, USA). Positive and negative controls sera were run in each test and a cut-off point was calculated by the mean of negative controls OD value plus two standard deviations.

Statistical analysis

Data were analyzed using SPSS software version 18 (Chicago, IL, USA). The association between asthma and *Toxocara* infection was determined using Chi-square test. Statistical analysis were also done to determine the association between seropositivity for toxocariasis and quantitative and qualitative variables using *t*-test and Chi-square test. A *P. value* of less than 0.05 was considered as statistically significant.

Results

The subjects of the study were 183 children aged 1-16 years old, consisting of 118 (64.5%) boys and 65 (35.5%) girls with the mean age of 7.55 ± 3.979 years (Table 1). Anti-*Toxocara* antibodies were detected in 9 out of 92 asthmatic patients (9.8%). ELISA test results revealed anti-*Toxocara* antibodies in 8 out of 91 (8.8%) healthy children. The asthma and control groups did not differ significantly in gender (*P*=0.079) and age (*P*=0.297).

This study indicates that there was no significant difference in *Toxocara* seropositivity between asthmatic and healthy children (*P*=0.817). Based on the statistical analysis, no significant association was found between IgG antibodies against *Toxocara* and variables such as gender (P=0.106) and age (*P*=0.243). The overall prevalence of *Toxocara* infection in the study population was 9.3%.

Analyses of *Toxocara* infection and variables among asthmatic and healthy children is shown in Table 2.

Table 1: Demographic data of asthmatic and healthy children

Study variables		***Asthmatic children N (%)***	***Healthy children N (%)***	***Total N(%)***	***P. value***
Gender	Male	65 (70.7)	53 (58.2)	118 (64.5)	0.079
	Female	27 (29.3)	38 (41.8)	65 (35.5)	
Age					
	1-5 years	28 (30.4)	38 (41.8)	66 (36.1)	
	6-10 years	40 (43.5)	24 (26.4)	64 (35)	0.297
	11-16 years	24 (26.1)	29 (31.9)	53 (29)	
Mean age		7.86±3.189	7.23±4.641	7.55±3.979	
Total		92 (100)	91 (100)	183 (100)	

Table 2: Analyses of *Toxocara* infection and variables among asthmatic and healthy children

Variable	*Seropositive for Toxocara N(%)*	*Seronegative for Toxocara N(%)*	*OR (95% CI)*	*P. value*
Gender				0.106
Male	14 (11.9)	104 (88.1)	2.782 (0.769-10.066)	
Female	3 (4.6)	62 (95.4)	1	
Age				0.243
1-5 years	7 (10.6)	59 (89.4)	-	
6-10 years	8 (12.5)	56 (87.5)		
11-16 years	2 (3.8)	51 (96.2)		
Groups				0.817
Healthy children	8 (8.8)	83 (91.2)	1	
Asthmatic children	9 (9.8)	83 (90.2)	1.125 (0.414-3.057)	
Total population	17 (9.3)	166 (90.7)	-	-

Discussion

Asthma is the most common chronic respiratory disease and its prevalence in children has been reported from 2% to 37% in different parts of the world (23). In Iran, the total prevalence of asthma was 10.9% (24). Due to the high prevalence of asthma especially in children, the identification of the risk factors that predispose the person to this disease is important. The parasites are considered as the risk factors associated with asthma. The effect of parasitic infections on asthma are determined by four factors; acute or chronic infection, intensity of infection, host genetics and parasite species (25). The allergic reaction to the larvae of *Toxocara* in lung causes pulmonary toxocariasis and *Toxocara* as an etiologic agent of asthma have been hypothesized (26, 27).

In Isfahan, central of Iran, the seroprevalence of *toxocariasis* was 45% in the asthmatics group and 21.7% in the control group (11). In another studies, anti-*Toxocara* antibodies were detected in the serum samples of asthmatic children, while no antibodies against *Toxocara* were detected in the control group (17, 20). These differences for the seroprevalence rate can be attributed to the weather and environmental condition, the rate of soil contamination with *Toxocara* spp. eggs in the studied areas, personal hygiene, the population studied and methods employed for diagnosis of toxocariasis. In our study, the seroprevalence of toxocariasis in asthmatic patients was higher than the healthy children, similar to other studies (14-16, 18, 19).

The findings of our study regarding the seroprevalence of toxocariasis in asthmatic and healthy children were consistent with the results of several studies where no significant difference in *Toxocara* seropositivity was observed between the two groups (11, 14-16, 19). In a study (18), the seroprevalence of toxocariasis was higher in asthmatic children as compared to the healthy control, but there was no statistically significant association between seropositivity to *Toxocara* and risk of asthma in children. In another study in Iran, seroprevalence of toxocariasis in asthmatic and healthy children aged 1-15 years old was compared, and the results showed that the seroprevalence of anti-*Toxocara* antibodies was 1.09% in the case group, while the control group was seronegative and there was no significant correlation between IgG antibodies against *Toxocara* and asthma (20).

In contrast to our study, several studies have reported a statistically significant difference between the seroprevalence of *Toxocara* infection among asthmatic and healthy children (6, 10, 11). Cobzaru et al (8) reported toxocariasis

in asthma patients and control group 68.42% and 13.63%, respectively, and this difference was significant. In addition, the seroprevalence rate of *Toxocara* infection was higher in asthmatic patients as compared to the healthy control and this difference was significant (11), while in our study, there was no significant difference in *Toxocara* seropositivity between the control group and asthmatic patients. In these studies, the report of a significant difference can be due to the higher prevalence differences of toxocariasis in asthmatic patients than the healthy persons.

Contrary to our results, Li et al (12) evaluated the association between asthma and toxocariasis using a systematic review and meta-analysis study, the results showed a significant association between *Toxocara* infection and asthma. Another systematic review and meta-analysis study reported that *Toxocara* infection is an important risk factor for childhood asthma (13).

This study was conducted on asthmatic, healthy children aged 1-16 years old, and there was no significant difference between the seroprevalence of toxocariasis and age. Our findings were consistent with the results of other studies (11, 17, 20, 28). In the current study, the seroprevalence rate of toxocariasis was higher in males than females, however, the statistical analysis showed no significant difference between *Toxocara* infection and gender. This is in keeping with the other study, which investigated the seroprevalence of toxocariasis in children and reported no significant association between toxocariasis and gender (10, 11, 17, 20).

One of the limitations of our study was the relatively low number of samples similar to many studied (8, 11, 17, 20). Another limitation was the lack of evaluation of some risk factors for acquiring *Toxocara* infection. However, the strength of this study include the diagnosis of asthma by a pulmonologist and good matching of asthmatic and healthy children in age and sex.

Conclusion

There was no significant association between toxocariasis and childhood asthma, also toxocariasis with age and sex. Further study on different regions such as urban and rural areas with a large sample size and using questionnaire for considering risk factors of asthma and toxocariasis is recommended.

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Conflict of interest

The authors declare that there is no conflict of interest.

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