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Content list available at: www.benthamopen.com/TOMICROJ/

DOI: 10.2174/1874285801610010162

RESEARCH ARTICLE

Detection of Specific Antibody Reactivity to *Toxocara* Larval Excretory-secretory Antigens in Asthmatic Patients (5-15 Years)

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Received: October 03, 2015 Revised: November 30, 2015 Accepted: December 01, 2015

Abstract:

Background & Purpose:

Humans act as an intermediate host for *Toxocara canis* and *Toxocara cati*. Toxocara may be an important risk factor for asthma in humans. The aim of the present study was to evaluate immunoglobulin G (IgG) anti-*Toxocara* canis antibody, using enzyme-linked immunosorbent assay (*ELISA*) in asthmatic patients (aged 5-15 years), referring to a clinic of pulmonary diseases in Arak, Iran.

Materials & Methods:

In this bi-group cross sectional study, serum samples were collected from 110 children with confirmed asthma and 70 children without asthma within one year. IgG anti-*Toxocara* antibody was detected *viaELISA* method. The collected data were analyzed, using SPSS.

Results:

The seroprevalence of antibodies against *Toxocara* species was estimated at 1.8% (two males) in asmathic children *viaELISA* method; however, no antibodies against *Toxocara* canis were detected in the control group. There was no significant correlation between the frequency of antibodies against *Toxocara* and variables such as age, gender, or place of residence (P>0.05). Moreover, the frequency of antibodies against *Toxocara* was not significantly correlated with contact with dogs, consumption of unwashed fruits and vegetables, or use of raw/undercooked sheep liver (P>0.05).

Conclusion:

The present study showed anti-*Toxocara* antibody in 1.8% of asthmatic children and determined the seroprevalence of toxocariasis in asthmatic children and adolescents in Arak, Iran. Based on the findings, the low rate of infection with *Toxocara* among asthmatic children may be attributed to acceptable personal hygiene and religious considerations.

Keywords: *Antibody*, *Asthma*, Asthmatic children, *ELISA*, Embryonated eggs, *Toxocariasis*.

INTRODUCTION

Toxocariasis in humans is caused by zoonotic nematodes of *Toxocara* genus. Humans act as an intermediate host for these parasites [1]. *Toxocara* infection commonly occurs in humans by the ingestion of embryonated eggs from soil, water, food, vegetables, and contaminated hands or direct contact with infected dogs and cats.

Toxocara cannot complete its life cycle in human hosts. The larva is released from the eggs in human intestines, enters the blood circulation, and eventually reaches various body tissues, particularly the brain, liver, lung, and eye

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tissues; as a result, the larva can grow to the second larval stage [2]. Children, given their poor personal hygiene, frequent contact with contaminated soil, and exposure to cats and dogs, are viewed as the most susceptible population to toxocariasis [3].

Glycan antigens are located on the glycoproteins of *Toxocara* larva and play an important potential role in stimulating Th2-type immune responses [4, 5]. These antigens are known to induce the production of immunoglobulin E (IgE) antibodies. The larvae are deployed in the lungs and cause an increase in inflammatory cells such as eosinophils, macrophages, and mast cells [6, 7].

Eosinophils cause the thickening of smooth muscle layers and lead to the narrowing of airways. In addition, eosinophils can produce free radicals, which induce vascular damage to the trachea, accompanied by pulmonary inflammation [5, 8]. Pulmonary symptoms, such as coughing, sneezing, fever, and breath shortness may occur in more than 50% of individuals infected with *Toxocara* [9].

Asthma is considered as a major chronic pulmonary disease throughout the world. The prevalence of this condition is reported to be 10% in adults and 0-30% in children, worldwide. Considering the diversity of risk factors for asthma, *Toxocara* infection may be regarded as an important risk factor for asthma in humans [9, 10]. Epidemiological knowledge about *Toxocara* can potentially assist specialists in patient treatment. The aim of the present study was to study the seroprevalence of toxocariasis in 5-15 years old asthmatic children, (aged), admitted to a clinic of pulmonary diseases.

MATERIALS AND METHODS

In this bi-group cross-sectional study, asthmatic children (aged 5-15 years), referring to a clinic of pulmonary diseases in Arak, Iran were studied during 2013-2014. The study sample included 110 asthmatic patients (case group) and 70 healthy subjects (control group).

Asthmatic patients were diagnosed by a pulmonologist. Patients with allergic or genetic asthma were excluded from the study. Based on the pulmonologist's diagnosis, all subjects in the control group were healthy in terms of pulmonary diseases.

Blood samples were collected from both groups, and serum samples were centrifuged for five minutes at the speed of 2500 rpm. The sera were stored at -20°C until further use and were then analyzed in order to detect IgG anti-*Toxocara* antibodies by enzyme-linked immunosorbent assay (*ELISA*) method. The commercial specific *ELISA* kits were purchased from IBL, Hamburg, Germany. The procedure was performed, according to the manufacturer's instructions.

Statistical Analysis

The collected data were used for descriptive and inferential statistics using SPSS version 16.0. For data analysis, Chi-square test was implemented to compare the groups and in all calculations P-values less than 0.05 was considered statistically significant.

Ethical Consideration

Informed consent forms were obtained from the subjects before enrollment in the study. They are ensured that the confidentiality of their information will be kept and on any circumstance the data will not disclosed to given to any third party.

RESULTS

The mean age of the subjects was 9.7 without any significant difference between the groups. The case group (n=110) consisted of 46 female (41.8%) and 64 male (58.2%) asthmatic children, while the control group (n=70) included 25 female (35.7%) and 45 male (64.3%) subjects. In the case group, 80% of the subjects resided in urban regions, while the remaining (20%) lived in rural areas. In the control group, 81.4% of the subjects resided in urban regions, while 18.6% lived in rural areas.

ELISA test results revealed the seroprevalence of IgG antibody against *Toxocara canis* in two asthmatic patients (1.8%), whereas no such antibodies were found in the serum samples of the control group (n=70). Based on the statistical analysis, the prevalence of IgG anti-*Toxocara* antibody was not significantly different between the two groups (P=0.5).

The results suggested no significant correlation between IgG antibodies against *Toxocara canis* and variables such as gender, age, place of residence, contact with dogs, consumption of unwashed fruits and vegetables, or use of uncooked sheep/cattle liver (Table **1**). Two patients with IgG anti-*Toxocara canis* antibody showed eosinophilia (average eosinophils: 12%). Moreover, there was no significant correlation between the increased level of eosinophils and the presence of IgG anti-*Toxocara canis* antibody (P=0.2).

Table 1. Demographic comparison between asthmatic patients with IgG anti-*Toxocara canis* antibodies and healthy individuals.

Variables		IgG against *Toxocara canis*		Chi-square test results
		Negative number (%)	Positive number (%)	
Age (years)	5-10	61 (100%)	0 (0%)	P=0.06
	11-15	47 (96%)	2 (4%)	
Gender	Male	62 (96.9%)	2 (3.1%)	P=0.4
	Female	46 (100%)	0 (0%)	
Place of residence	Urban areas	77 (97.5%)	2 (2.5%)	P=0.8
	Rural areas	21 (100%)	0 (0%)	
Contact with dogs	Yes	9 (100%)	0 (0%)	P=0.1
	No	99 (98.1%)	2 (1.9%)	
Consumption of unwashed fruits and vegetables	Yes	28 (93.4%)	2 (6.6%)	P=0.1
	No	80 (100%)	0 (0%)	
Consumption of undercooked cattle or sheep liver	Yes	11 (100%)	0 (0%)	P=0.5
	No	97 (98%)	2 (2%)	

DISCUSSION

In the present study, there was no significant relationship between the seroprevalence of anti-*Toxocara* antibodies and gender, age, or place of residence in asthmatic children (5-15 years old referring to a clinic of pulmonary diseases in Arak, Iran.

Ample evidence suggests that helminthic parasites may cause asthmatic and allergic attacks in humans, the intensity of which depends on the number of parasites and host species, as well as some other genetic factors [11, 12]. For instance in study of Cobzaru and colleagues on 76 patients aged 5-16 years, An *ELISA* test based on the detection of anti-Toxocara canis (E/S antigen) serum IgG and E was done in groups (with or without asthma), their findings showed that seroprevalence in asthma patients was 68.42% and in the controls 13.63% and this difference was significant [13].

Humans can be accidental intermediate hosts for *Toxocara* species. These parasites cannot reach maturity in the human body and *Toxocara* larvae can be distributed in any body tissue, even inside the lungs. *Toxocara* larvae, due to their superficial glycan antigens and production of excretory-secretory antigens, induce Th2-type immune responses [4, 5].

The study of the lungs of mice, infected with embryonated eggs of *Toxocara*, indicated tracheal inflammation [14]. In addition, based on previous research, with increased level of eosinophils in the lungs, the tracheal smooth muscle layers are thickened and cause the narrowing of trachea; this eventually leads to the development of asthma in these individuals [5, 15].

Based on the findings of the present study, the prevalence of anti-*Toxocara* antibodies in asthmatic children was estimated at 1.8%, which was lower than the rates reported by Chan in Malaysian children (6.8%) and Fernando in Sri Lankan children (29%) [9, 16]. Comparison of previous research and the present study revealed the contribution of various factors to the low rate of infection in this study, including differences in eating habits among Iranians and religious beliefs concerning pets (*e.g.*, dogs). In fact, pets including dogs are considered unclean in religious terms and direct and close contact with them is prohibited.

The main findings of the present study about the lack of relationship between seroprevalence of anti-*Toxocara* antibodies and gender, age, or place of residence was consistent with the results reported by Mosayebi, Alavi, Sadjjadi, and Kustimur and in contrast with the findings reported by Santos [17 - 21]. Although there was no significant relationship between gender and toxocariasis in the present study, two male patients were contaminated, which is probably due to the characteristic behaviors of boys and the games they play [22].

The high incidence of toxocariasis in children can be also due to their poor personal hygiene, soil contamination,

and ingestion of soil materials contaminated with parasite eggs; however, as children grow, they are less exposed to these eggs. In this study, the individuals infected with *Toxocara* resided in urban areas. However, in the majority of conducted studies, infection has been reported in individuals living in rural areas, which can be attributed to their contact with dogs, as well as the contaminated environment in these areas [23, 24].

In parasitic infections, direct demonstration of adult worms, larvae, or eggs can make a definite diagnosis. Nevertheless, it is complicated to find out the larvae of T. canis in the tissue by biopsy due to extensive distribution and small size of larvae. Moreover biopsy is an invasive diagnostic method. In this context, serology is an alternative method for diagnosis of toxocariasis [25]. The most common serodiagnosis approach is *ELISA* using specific antigens. But there are some issues, first infection with parasites *e.g*. toxocara might be ignored. For example, Lim and his colleagues [26] suggested that toxocariasis is common but neglected by doctors mostly due to their lack of awareness. The second, toxocariasis is unaware to doctors and abandoned by lack of good routine diagnostic service in many regions [27]. The seroprevalence of anti-*Toxocara* antibodies was not significantly associated with the prior history of contact with dogs, consumption of unwashed fruits and vegetables, or use of cattle/sheep liver ($P>0.05$). The patients with antibodies against *Toxocara* had a previous history of contact with dogs; this finding was in agreement with the results reported by Mosayebi, Chan, and Fernando [9, 16, 17], although due to small rate (two cases) this findings is not statistically useful.

Given the fact that *Toxocara* infection in dogs is reported to be as high as 60% in Iran, contact with these animals can be considered as a risk factor for toxocariasis [28]. In Egypt, consumption of raw vegetables contaminated with *Toxocara* eggs was reported to be 19%. Also, use of unwashed fruits and vegetables could increase the risk of contamination with *Toxocara* [29].

In the present study, two patients had a prior history of eating unwashed fruits and vegetables, while they had not consumed undercooked cattle or sheep liver. This finding was in correspondence with the results reported by Mosayebi, though in contrast with the study by Kwon on children in Korea [17, 30].

Based on the majority of conducted surveys, increased eosinophil count is associated with a rise in anti-*Toxocara* antibodies [17, 26, 31, 32]. In the present study, just two patients suffered from hypereosinophilia. However, there was no significant relationship between the seroprevalence of anti-*Toxocara* antibody and the increased level of eosinophils ($P>0.05$); this can be attributed to the small sample size in the present study. The increased eosinophils, however, can be a common finding in the study of *Toxocara* infection [9].

CONCLUSION

Based on the results of the present study, the lower rate of *Taxocora* infection among asthmatic children in Arak, Iran may be due to personal hygiene or religious considerations, prohibiting any contact with dogs. Further study on different age groups with a larger sample size is highly recommended.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Hereby, we would like to express my gratitude to the members of Parasitology, Mycology, and Entomology Departments, the staff at Dr. Qasi Saeedi Laboratory, and internist, Dr. Hamidreza Ahadi. This study was based on a research project (No. 832), approved by the Research Council and Ethics in Research at the Faculty of Medicine, Arak University of Medical Sciences. We would also like to thank all the members of the council for their cooperation.

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