Toxocara infection and childhood allergic asthma, a Case-Control Study in Boyer Ahmad County; southwest Iran

Ali Pouryousef[1], Samer Mehrabi[2], Reza Abbasi[2], AbdoAli Moshfe[2], Fattaneh Mikaeili[3], Zahra Rezaei[4], Davoud Rostamzadeh[2], Amin Alah Saadat[2], and Nasir Arefkhah[2]

[1]Sabzevar University of Medical Sciences
[2]Yasuj University of Medical Sciences
[3]Shiraz University of Medical Sciences School of Medicine
[4]Shiraz University of Medical Sciences Professor Alborzi Clinical Microbiology Research Center

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Introduction

Allergic asthma is the most common chronic disease in different communities ((1). On average, hundreds of millions people have been suffered from allergic asthma at least once (1). The increasing prevalence of this disorder worldwide has imposed considerable pressure on the healthcare system, especially in developing countries (2). Exposure rate and type of inhaled allergen play an essential role in developing allergic asthma symptoms (1). Although genetics, food, pollen, house dust mites, and chemicals all have a role in the onset of allergic symptoms, there is growing evidence that human immune responses to parasitic worm glycan antigens may influence the development of allergy symptoms. This statement is defensible since in response to helminth infections high levels of total and specific IgE antibodies are produced which cause mast cells to degranulate, releasing vasoactive substances, including histamine, and also Th2 pathway responses are activated that are characterized by the cytokines production including IL-4, IL-5, and IL-13 (3).

In the same direction, strong evidence from experimental and clinical studies has documented that human toxocariasis (HT), a parasitic zoonotic disease caused by Toxocara spp., afflicts humans as an incompatible host in the parasite's life, might be linked with childhood asthma (4). In addition, some studies showed a correlation between Toxocara spp. infection and development of allergy in human individuals and animal studies (5).

The high prevalence of human toxocariasis in tropical countries has sounded the alarm calling to prevent and control this infection, especially among children (6). Children are significantly more prone to Toxocara infection due to poor personal hygiene and habits such as geophagy, finger sucking, and nail biting (7). Therefore, studying toxocariasis in childhood asthmatics gained much attention in places where toxocariasis and childhood allergic asthma is a big challenge for the healthcare system. Due to the lack of comprehensive study on toxocariasis as a potential risk factor in children, as well as its possible association with the etiology of allergic asthma in southwest Iran, this study aimed to compare the seropositivity rate of anti-Toxocara IgG in children with allergic asthma and healthy children.

Materials and Methods

Study area: Boyer-Ahmad County, with over 300,000 population, is located in Kohgiluyeh and Boyer-Ahmad Province in southwestern Iran. The geographical coordinates of this region are between 30* 40' 5.66" N and 51* 35' 16.66" E. The high average annual rainfall in Boyer-Ahmad County (almost 354 mm) has

made this area one of Iran's most important hubs of agriculture and animal husbandry (8).

Patients and Sampling: This case-control study was conducted in Boyer-Ahmad County in southwest Iran between June to October 2021. Two hundred allergic asthma children (aged 1-15 years) admitted to Emam Sajjad Hospital in Boyer-Ahmad County, having at least one allergic asthma manifestation, whom a pediatrist diagnosed according to the International Study of Asthma and Allergies in Childhood (ISAAC) criteria (9). The diagnosis of asthma was made if all the following criteria were met: (I) Recurrent episodes of one or more of the following symptoms : wheezing, cough, breathing difficulties, and chest tightness, particularly at night or in the early hours of the morning; (II) Respiratory symptoms improve spontaneously or after treatment (bronchodilators associated or not with corticosteroids); (III) Presence of triggers or aggravating factors such as exposure to allergens or irritants, physical exercise, weather changes or emotional stress. Individuals with a genetic predisposition to allergic asthma were excluded.

Furthermore, 208 children were enrolled as healthy subjects with non-allergic conditions based on the above-mentioned criteria. Control subjects were individually matched regarding age, sex, and residence. Five mL of fresh blood was taken from each individual, and the sera were separated and stored at -20°C until serological tests were performed. The informed consent form, along with a structured questionnaire containing sociodemographic information and related risk factors for toxocariasis such as close contact with dog or cat, \RL pica disorder, consumption of unwashed vegetables or contaminated water, allergic asthma risk factors including parental asthma, as well as the contact with soil was questioned and filled up by parents of participants.

ELISA

The native excretory-secretory (E/S) antigen from the second-stage larvae of Toxocara canis was used to perform a manual enzyme-linked immunosorbent assay (ELISA) previously prepared (Zibaei et al. 2016). A brief of the ELISA method was as follows: 5 µg/ml of antigen in carbonate bicarbonate buffer (pH 9.6) was coated in 96 wells of microplate and incubated at 4°C. The next day, the microplates were automatically washed by ELISA washer with washing buffer (containing 0.05% Tween 20 in PBS). 5% skimmed milk in PBS was added to each well and incubated for 2 hours at room temperature to eliminate non-specific surface attachment and improve assay sensitivity. After washing according to the method, 100 µl of diluted serums (1:100 in PBST solution) were poured into the wells. Next, after washing, 100 µl of secondary antibody anti-human IgG conjugated with horseradish peroxidase (1:4000 in PBST) was added to ELISA plate wells and then incubated in a dark place at 37 °C for one hour. The plate was then washed as before, and 100 µl of the substrate (containing 0.4 mg/ml OPD, 0.3% H2O2 in 0.1 M citrate buffer, pH=5.6) was added to it then; the plate was placed in a dark place for 15 to 20 min at room temperature to visualize the antigen/antibody reaction. Finally, the stopping solution (sulfuric acid) was added (100 µl/well) to stop the reaction, and then the OD values were measured at an absorbance of 490 nm by an ELISA plate reader (ELx800, BioTek, USA). Each test included positive and negative controls, and the cut-off point was determined using the following formula: the mean of the negative controls' OD value plus two standard deviations. Also, all positive sera were double-checked for final confirmation by the described ELISA method.

Statistical analysis

Statistical analyses were performed using SPSS software version 20 (IBM, Armonk, NY, USA). The Chi-square test was used to test the significance between the two groups. To identify the relationship between Toxocara seropositivity and asthma status, the Chi-square test and logistic regression were used to determine odds ratios (ORs) and 95 percent confidence intervals (CIs). The value < 0.05 were considered statistically significant.

Results

A total of 408 participants, including 200 patients with asthma as a case group with a mean ± SD age of 8.19 ± 3.2 years and 208 individuals without asthma as a control group with a mean ± SD age of 7.79 ± 3.4 years, were included in the study. According to the statistical test, both patient and control groups did

not differ significantly in age, location, and sex distribution. (P [?] 0.05) **(Table 1)** .

The prevalence of Toxocara infection in the study population (patients and control individuals) was 6.9% (95% CI, 3–5.62%; 28/408). Sixteen out of the 200 children with asthma (8%; 95%CI, 2.11–6.49%) and 12 out of the 208 children without asthma (5.8%; 95%CI 2.71–5.9%) were seropositive for anti-Toxocara IgG antibodies. There were no statistically significant differences in the prevalence of toxocariasis between children with asthma and children without asthma (OR, 1.420; 95% CI, 0.654-3.083; P value = 0. 373) (Table 2).

In subgroup analyses based on sociodemographics, Toxocara infection seropositivity was significantly associated with asthma in children whose parents were farmers (OR, 5.647; 95%CI, 1.168-27.299; P-value = 0.017), and those with frequent contact with soil (OR, 3.225; 95%CI, 1.044-9.961; P-value = 0.033). More details and significance for other variables are shown in Table 3.

The multivariate analysis showed that a history of atopic asthma in the family (OR, 3.463; 95% CI, 2.005-5.982; P-value < 0.001), soil contact (OR, 2.658; 95% CI, 1.675- 4.218; P-value < 0.001), and eating unwashed (raw) vegetables (OR, 2.575; 95% CI, 1.454- 4.558; P-value < 0.001) were independent factors associated with asthma (Table 3). Additionally, no significant association was found between seropositivity to Toxocara spp. and asthma.

Discussion

The prevalence of asthma in children has been reported from 2% to 37% in different parts of the world (10). The prevalence rate of childhood asthma has been reported to be 10.9% in Iran (11). Identifying the risk factors that can affect allergy and asthma is crucial. Some previous experimental and epidemiological studies have suggested that geo-helminth infection, such as toxocariasis, might play an etiological role in developing asthma and other allergic disorders(4, 12) However, according to the hygiene hypothesis, this relationship is still controversial and awaits to be explored (13). This investigation discovered that children with asthma had a greater seroprevalence of *Toxocara* infection than age- and gender-matched controls, showing that *Toxocara* seropositivity was not an independent risk factor for developing asthma in this pediatric population in Boyer-Ahmad Province.

Regarding the seroprevalence of toxocariasis in asthmatic and healthy children, the present study was in agreement with some previous epidemiologic studies where no significant difference in *Toxocara* seropositivity was observed between the two groups (14, 15). In contrast to our study, several studies have reported a statistically significant difference between the seroprevalence of *Toxocara* infection among asthmatic and healthy children(16-19). In a study by Cobzaru et al., the seroprevalence of toxocariasis in asthmatic children (68.42%) was significantly higher than in the control subjects (13.63%) (16). Moreover, two published meta-analysis studies in 2014 and 2018 indicated that *Toxocara* infection could be a potential risk factor for developing asthma (20, 21). An explanation for these different results could be attributed to the age of the study population, geographic and environmental conditions in each region, the rate of soil contamination with *Toxocara* spp. eggs in the studied areas, parasite load, personal hygiene, study design, and different sensitivity and specificity methods used to diagnose toxocariasis (commercial ELISA or Western blot). Although there was no significant difference in the prevalence of toxocariasis between children with and without asthma in this study, asthmatic children who had frequent contact with soil (one of the most important known risk factors for toxocariasis) and those whose fathers were farmers had significantly higher *Toxocara* seropositivity than healthy children. These findings indicate that this region of Iran is contaminated with *Toxocara* eggs, and the risk of acquiring infection is high, particularly for children who frequently play in similar conditions in the southwest of Iran. Our study also found that children with a history of atopic asthma in the family, soil contact, and eating unwashed raw vegetables were more susceptible to having asthma. Some studies confirm a positive association between these underlying factors with asthma (22-24). In the present study, the prevalence of toxocariasis in the total study population (asthmatic and non-asthmatic children) was 6.9%. Several case-control studies had a higher overall prevalence than our results (12, 16, 25). For example, in two studies by Momen et al. (in Iran) and Cobzaru et al. (in Romania), the seroprevalence of toxocariasis

in the study population was reported to be 33.35.% and 41%, respectively (12, 16). The fact that most of the children in our study lived in the city and their lifestyle was such that the majority had no history of exposure to dogs and cats may explain the lower overall prevalence of toxocariasis in our study compared to most other studies.

On the contrary, some studies report a lower prevalence of toxocariasis than the current study. In this regard, Darvish et al. and Sadri et al. reported the prevalence of toxocariasis at 2.48% and 1.09%, respectively, which may be due to the use of different serological diagnosis methods and antigenic sources(17, 26). A relatively high prevalence of HT is seen in children (6.9%) in current study. Therefore, control and preventive measures against toxocariasis must be considered part of the study region's healthcare system policies. Some suggested preventive strategies are public education of parents about ways of transmitting the infection, proper fencing around farms and children's playgrounds, and deworming of dogs and cats. The most critical limitations were the small sample size and the absence of confirming methods for definitive toxocariasis diagnosis (such as Western blotting). Despite the mentioned limitations, using native secretory-excretory antigen (ES) with acceptable sensitivity and specificity significantly reduces cross-reactions (Barr et al. 2014). In a study done by Zibaei et al. to diagnose toxocariasis, the sensitivity and specificity of ELISA using *T. cati* ES antigens were 97.0% and 96.7% respectively (27). Accurate diagnosis of asthmatic children by an experienced pediatrician in allergy and immunology based on the ISAAC questionnaire and good matching of groups in terms of age, gender, socioeconomic status, and place of residence were among the strengths of our study.

Conclusion

It was important to note that 6.9% of the children were seropositive for Toxocara. Although our study did not find a significant association with childhood asthma, toxocariasis is a severe threat to children who have contact with soil in the studied area. Therefore it is necessary to establish programs to prevent this neglected tropical disease. We recommend control measures for toxocariasis through health education regarding preventing young children from lying in contaminated soil. It is suggested that more studies on regions such as urban and rural areas with a large sample size be conducted to investigate the association between toxocariasis and childhood asthma.

Ethical approval

This study was approved by the research ethics committee of the Yasuj University of Medical Sciences Medical Ethics Committee (ethical code: IR.YUMS.REC.1400.126).

Consent to participate

Written informed consent was obtained from the parents.

Authors' Contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by AP, SM, RA, DR, AAS, FM, AAM, ZR and NAK. Nasir Aref Khah and Ali Pouryousef wrote the first draft of the manuscript, and all authors commented on previous versions. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are included in the study.

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Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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