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Frequency of Toxocariasis Among Asthmatic Children in Northeastern Iran

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Abstract

Background: Toxocariasis is a zoonotic and telluric disease caused by the *Toxocara* species mostly in tropical areas. The relationship between toxocariasis and asthma has always been a subject of discussion.
Objectives: This study evaluated the seroepidemiology of *Toxocara* among asthmatic children.
Methods: This cross-sectional study evaluated 150 children aged 3 - 12 years with asthma presentations, who were referred to Dr. Sheikh Hospital of Mashhad University of Medical Sciences from April 2017 to March 2018. Serum samples were tested for the presence of anti-*Toxocara* antibodies using Enzyme-linked Immunosorbent assay (ELISA). Positive sera were confirmed by the Western Blotting (WB) method.
Results: Out of 150 asthmatic patients, *Toxocara* immunoglobulin G (IgG) antibody responses were observed in two (1.3%) patients by ELISA and one (0.6%) patient by both ELISA and WB. Moreover, none of the patients was detected as hypereosinophilia.
Conclusions: It seems there is no significant relationship between *Toxocara* infection and asthma in Northeastern Iran. These findings suggest the need for WB immunodiagnosis and ELISA using *Toxocara* antigens to improve human toxocariasis diagnosis in patients with asthma.

Keywords: Asthma, Children, ELISA, Toxocara, Western Blotting, Antibody

1. Background

Toxocariasis is among the neglected zoonotic diseases with a worldwide incidence. Humans can be infected with this parasite through the accidental ingestion of eggs or the consumption of contaminated raw and undercooked meat (1). The larvae hatch in the small intestine and migrate through somatic organs, preferably the liver, brain, and eyes. They can cause visceral larva migrans (VLM), ocular larva migrans (OLM), neurotoxocariasis, and covert toxocariasis (CT) (2).

Asthma is a chronic disease that causes significant morbidity. Pediatric asthma is one of the most common conditions causing emergency department visits, hospitalizations, and missed school days in childhood (3, 4). There is a question that how a *Toxocara* larva stimulates respiratory reactions in humans. The rate of excretory-secretory antigens (ES Ags) secreted from each larva is 2 ng per day. The TBA-1 is introduced as the most allergic fraction of ES proteins (5). Chronic cough was presented in volunteers who had ingested 100 embryonated eggs of *T. canis* (6).

The test for the immunodiagnosis of toxocariasis includes Enzyme-Linked Immunosorbent assay (ELISA) using *Toxocara canis* ES Ags (5, 7). As a result of cross-reactions with some nematodes such as *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale*, and *Strongyloides stercoralis*, the Western Blotting (WB) technique should be used to confirm the diagnosis due to its much higher specification (8).

The association between toxocariasis and childhood asthma is still unclear. Limited epidemiological and clinical studies have reported the correlation between *Toxocara* infection and asthmatic children in Iran (9).

2. Objectives

Some studies advocate this opinion while others reject it. Thus, a cross-sectional study was performed to find the *Toxocara* antibody in asthmatic children in a pediatric hospital located in the northeast of Iran.

3. Methods

3.1. Subjects Population

Blood samples were collected from 150 asthmatic children aged 3 - 12, referring to Dr. Sheikh Hospital, during 15 months from April 2017 to March 2018. All children had a history of asthma for at least six months. The consent of parents was obtained. Then, 3 mL of blood was collected from each child. The serum was separated and stored at -70°C until use. The complete blood count (CBC) was prepared and eosinophil count was calculated individually. The risk factors for asthma and *Toxocara* infection were recorded in a questionnaire given to the parent or legal guardian of each child.

3.2. Serological Tests

3.2.1. ELISA

All serum samples were analyzed for IgG antibody production against *T. canis*, using an ELISA kit (product number: TOCG0450, Dietzenbach, Germany). This ELISA kit had high specificity and sensitivity (> 95%). The ELISA procedure was performed according to the manufacturer's instructions. The result was read using an ELISA reader, equipped for absorbance measurement at 450/620 nm. According to the manufacturer's instructions, an index positivity of > 11 NTU (NovaTec Units) was considered as positive and < 9 NTU as negative for *T. canis* infection.

3.2.2. Western Blotting

Positive serum samples were investigated by confirmatory WB analysis, using a commercial kit (LDBIO Diagnostic, Lyon, France). First, sera were diluted at 1:100. After the incubation of strips (2 h at room temperature), a washing step was performed at least three times in PBS containing 0.1% Tween 20. The strips were incubated again for 2 h at room temperature with the second antibody, anti-human immunoglobulin G peroxidase conjugate (dilution 1:1,000). After another washing step similar to the previous protocol, the diaminobenzidine substrate was added. Distilled water was used to stop the reaction. The results were considered as positive when the samples reacted to two or more low-molecular-weight bands (LMWB; 24 - 35 kDa).

4. Results

Based on the results obtained by ELISA, two (1.3%) children with a history of asthma exhibited antibodies against *Toxocara*. Regarding the eosinophil count, the two mentioned cases were in the normal range. Eosinophil blood counts were 1% and 3% in the two patients.

Serum samples that were positive for anti-*Toxocara canis* antibodies by ELISA were investigated using confirmatory WB analysis. The positive WB strips indicating the presence of specific anti-*Toxocara* IgG in the samples showed at least two or more bands between 24 and 35 kDa (Figure 1). Anti-*Toxocara* antibodies were detected in one (0.6%) patient with positive ELISA.

5. Discussion

Asthma in children and adults has a prevalence rate of 35.4% in Iran, the highest among Middle East countries (10). Twenty-eight studies from 19 provinces of Iran showed 2.7% and 3.5% asthma prevalence in children aged 6 - 7 and

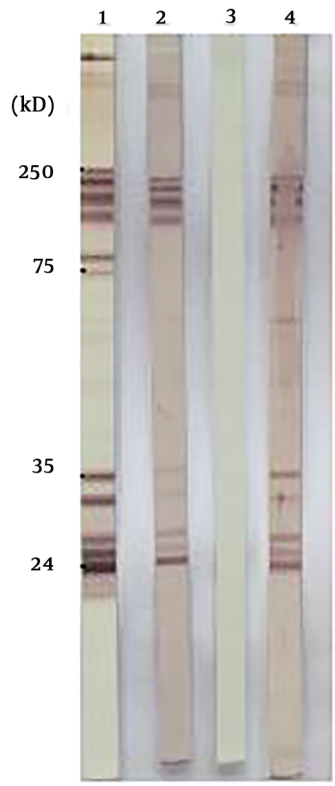

Figure 1. Western blotting with *T. canis* ES protein. 1, Molecular weights marker; 2, positive control; 3, negative control; 4, *Toxocara* patient. Sera samples reacted with no band.

13 - 14 years, respectively (3). Many risk factors can affect allergic and asthmatic diseases. Recently, strong evidence has been published stating that *T. canis* is an efficacious factor for childhood asthma. However, it is controversial and remains unclear, as the number of articles confirming this positive association (11, 12) is the same as the number of articles conflicting with this hypothesis (13, 14). A meta-analysis study found a highly significant association between *T. canis* infection and asthma in 723 cases and 807 controls by an odds ratio of 3.36. Also, in a cross-sectional study, the odds ratio of toxocariasis was 1.73% in asthmatic children (15). Recently, in a case-control study in central Iran, the seroprevalence of *Toxocara* antibodies was 45% in asthmatic patients and 21.7% in controls (P = 0.02) (16).

Interestingly, toxocariasis has a high prevalence in dogs in Iran (9). In a review of 26 studies carried out between 1997 and 2015, the prevalence of toxocariasis in carnivores was reported to be 18.81% in Iran (17). The rate of dogs with *Toxocara* was reported to be 4.4% to 7% in this area (18, 19). Moreover, the ELISA technique has shown more *Toxocara* seropositivity in pet owners than in the control group (20.43% and 1.07%, respectively) in this region (20). The first population study based on serological findings in Iran was reported by Sadjjadi et al. (21) using the sera of 436 clinically healthy children. It showed that 25.6% of the examined subjects were positive for *Toxocara* ES Ags (21). Therefore, this nematode is prevalent in Iran although it is not a significant or contributory cause of asthma.

In this study, only 0.6% of asthmatic children suffered from toxocariasis without hypereosinophilia (22). Around 25% of children with visceral larva migration had no eosinophilia. In covert toxocariasis, the number of eosinophilia was reported to be lower on average (23, 24).

The present study did not find any significant prevalence of *Toxocara* infection among 150 asthmatic children that is consistent with some other studies (25, 26). In another study, the number of seropositive asthmatic children was almost equal to the control group (27). The limitation of this study was that it had not matched the control group.

The ELISA is the primary screening test for the diagnosis of visceral toxocariasis and WB is a confirmatory method (28). Unlike the fact that available serodiagnostic tests used for toxoplasmosis can determine the approximate onset of infection, the serodiagnostic tests used for *Toxocara* cannot distinguish between the past and current toxocariasis infections. The disadvantage of the ELISA technique for the diagnosis of toxocariasis is a cross-reaction with some intestinal nematodes. Thus, ELISA-positive samples must be confirmed by WB to remove false positives (5).

Determining the substantiate role of toxocariasis in asthma disorder is challenging due to the fact that there are various causes for this disease. Further studies are required to clear this correlation. However, if toxocariasis did not cause asthma directly, it could act as a co-factor to increase the severity of the symptoms of bronchial asthma (27).

5.1. Conclusions

This study found no significant relationship between the prevalence of toxocariasis and asthma. Factors such as keeping dogs or the presence of pica can be helpful in prescribing ELISA for the diagnosis of toxocariasis in asthmatic children. It is suggested that more observational studies be conducted to investigate the association between toxocariasis and childhood asthma.

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Footnotes

Authors' Contribution: Study concept: Seyed Aliakbar Shamsian. Selection of patients: Mohammad Javad Sayedi. Manuscript drafting: Elham Moghaddas. ELISA performance: Soheila Vaghei. Western blotting performance and critical revision of the manuscript: Mohammad Zibaei.

Conflict of Interests: None.

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Informed Consent: Written informed consent was obtained from all patients' parents.

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