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The association between seropositivity to human toxocariasis and childhood asthma in northern Iran: a case-control study

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Abstract
Background: Besides the well-known risk factors, *Toxocara* infection is thought to play a significant etiological role in the development of childhood asthma. To further explore this association, the prevalence of *Toxocara* infection in sera of asthmatic children and healthy controls in northern Iran was investigated.
Methods: In this case-control study, cases were 145 physician-confirmed asthmatic children diagnosed according to the Global Initiative for Asthma (GINA) guidelines. Controls were 115 age-sex-residence-matched children who did not have physician-diagnosed asthma. The presence of anti-*Toxocara* immunoglobulin G (IgG) was tested using enzyme-linked immunosorbent assay. Univariate and multivariate logistic regression methods were used for case-control comparisons.
Results: Seropositivity rate was 4.1% (95% CI, 3.4-4.7%) in asthmatic children and 0.86% (95% CI, 0.71-1.0%) in controls, suggesting a strong association (*P*-value < 0.02). Moreover, *Toxocara* infection was not significantly more prevalent (*P*-value = 0.12) in children with moderate sustainable asthma (9.3%, 3/32) than in children with mild sustainable asthma (2.3%, 3/113). Mean total immunoglobulin E (IgE) level was significantly higher in *Toxocara*-infected children (222.3 ± 367.1) than in non-infected children (143.19 ± 218.05) in the case group (*P*-value < 0.05).
Conclusions: Our findings indicated that *Toxocara* infection can play an important role in childhood asthma. Further experimental and epidemiological studies are needed to clarify this hypothesis.

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Introduction

Toxocariasis, a worldwide distributed human parasitic disease, is caused by two species of nematode worms from ascaridoid family, *Toxocara canis* and *Toxocara cati*.[1,2] These parasites are common intestinal worms of canine and feline.[3,4] Humans are paratenic hosts and infection occurs accidentally by the ingestion of infective eggs or, less frequently by contaminated meats containing infective larvae.[5,6] Although the majority of human cases of toxocariasis are asymptomatic, larval migration to the liver, lungs, brain, and other organ systems can result in several syndromes known as visceral larva migrans (VLM), ocular larva migrans (OLM), neurotoxocariasis (NT), and covert or common toxocariasis.[5,7] Additionally, in recent years, a growing body of investigations have suggested that human toxocariasis could be a potential risk factor to the development of allergic disorders, such as asthma, atopy, rhinitis, urticaria, and eczema.[5,8-10]

Asthma is a common inflammatory allergic disease affecting around 300 million people worldwide. In 2017, it was estimated that globally, asthma could be responsible for 4,95,100 deaths and about 23 million disability-adjusted life-years.[11,12] At the global level, almost one in 10 children and one in 12 adults is affected by asthma, and the disease is the cause of a very huge annual healthcare expenditure.[13] Two forms of asthma (allergic and non-allergic) have usually been defined in the clinic, and children are frequently affected by allergic asthma.[13] The exact causes of childhood asthma are not well defined, although it is indicated that both genetic (e.g., family atopic history) and environmental factors (e.g., parental smoking, indoor and outdoor allergens, air pollution and infections) are involved in the onset of childhood asthma. Moreover, some evidence from experimental and clinical studies suggests that *Toxocara* infection can promote the onset of allergic diseases such as asthma.[14-16]

Mazandaran province located in the north of Iran is an endemic area for many zoonotic parasites.[17-19] A recent study showed that approximately 13% of children in this area are seropositive for *Toxocara* infection.[20] A previous study also showed about 4% of children in this area to be affected by asthma symptoms.[21] To our knowledge, there has been no study to evaluate the association between *Toxocara* infection and childhood asthma in this area. Therefore, we designed the present study to assess this association and also to determine the possible risk factors for *Toxocara* infection in this area.

Ethical approval

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participant's parents included in the study.

Methods

In this case-control study, we recruited 145 asthmatic children (55.2% male, mean age 6.88 ± 2.58 years) and 115 healthy controls (56.5% male, mean age 6.62 ± 3.63 years) referred to the asthma and allergy clinic at Amirkola Hospital, the largest referral pediatric hospital in Mazandaran province, north of Iran.[22] The study protocol was approved by the Research Ethics Committee of the Babol University of Medical Science, Babol, Iran (no. IR.MUBABOL.HRI.REC.1396.207). Asthmatic children (cases) were recruited according to the following inclusion criteria: physician-diagnosed asthma based on Global Initiative for Asthma (GINA) criteria,[23] age 2–15 years, and designed consent form was filled in by their parents or legal guardian. Control subjects were non-asthmatic children with the same criteria as the case patients. Children were excluded if they had used anti-parasitic drugs in the past 6 months, or had known clinical symptoms related to toxocariasis such as hepatosplenomegaly, generalized lymphadenopathy, and ocular symptoms. Further details about participation and inclusion and exclusion criteria have been presented in our previous publication.[22] The parents were asked to complete a questionnaire containing questions about risk factors for asthma, including family atopic history, parents smoking in the home, the child's contacts with pets, and annual family income; and also risk factors for *Toxocara* infection, including dog or cat ownership, contact with soil, use of unwashed vegetables, use of undercooked meat, and drinking of unsafe water.

For each participant, a venous blood sample of 5 mL was drawn through venipuncture. Blood samples were centrifuged at 2500 rpm for 15 min. Sera were separated in sterile tubes and were then transported in ice to the laboratory of the Infectious Diseases and Tropical Medicine Research Center at Babol University of Medical Sciences, where they were stored at -20¤C until use. All collected sera were examined for anti-*Toxocara* immunoglobulin G (IgG) antibodies using a commercial ELISA kit (NovaTec Immunodiagnostics, Dietzenbach, Germany). The sensitivity and specificity of this kit was more than 95%. All procedures were performed as per the manufacturer's instructions. The serological examiner was blind to the child's asthma status and about controls. We considered samples of <9.0, 9–11, and >11.0 IU/mL as negative, suspicious, and positive, respectively, as recommended by the manufacturer.

Statistical analyses were performed using SPSS Statistics software, version 21 (IBM, Armonk, NY, USA). The seroprevalence of *Toxocara* infection in each group is described as the relative percentage with an exact binomial 95% confidence interval (CI). The univariate analyses and multivariate model by Penalized Logistic Regression (PLR) method were used to calculate the Odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between asthma and *Toxocara* infection and also to identify the asthma risk factors and *Toxocara* infection risk factors. A *P*-value <0.05 was considered statistically significant.

Results

The main socio-demographic features of asthmatic and healthy children are presented in Table 1. The overall

seroprevalence of *Toxocara* infection in the study participants was 2.7% (95% CI, 2.4– 2.9%; 7/260) with a seropositivity rate of 4.1% (95% CI, 3.4–4.7%; 6/145) in asthmatic children and 0.86% (95% CI, 0.71–1.0%; 1/115) in healthy controls.

Adjusted ORs determined through univariate and multivariate analyses for asthma risk factors were also presented in our previous publication.[22] In this study, we also include three further items (family atopic history, eating unwashed vegetable, and water source) in multivariate

Table 1 Demographic characteristics of participants and multivariate analyses of asthma risk factors among case-patients and controls.

Variable	Children with asthma (n=145)		Children without asthma (n=115)		Adjusted OR (95% CI)	P-value
	N	%	N	%		
Sex						
Male	80	(55.2)	65	(56.5)	1	0.951
Female	65	(44.8)	50	(43.5)	0.9 (0.4-2.2)	
Age						
≤6	73	(50.3)	59	(51.3)	1	
7-12	70	(48.3)	48	(41.7)	1.1 (0.5-2.7)	0.69
13-18	2	(1.4)	8	(7.0)	0.7 (0.1-5.1)	0.78
Residence						
Urban	99	(68.3)	60	(52.2)	1	
Rural	46	(31.7)	55	(47.8)	0.3 (0.1-0.9)	0.04
Family income						
≥1,500,000 T	58	(40.0)	32	(27.8)	1.06 (0.44-2.5)	0.89
<1,500,000 T	87	(60.0)	83	(72.2)	1	
Dog contact						
Yes	8	(5.5)	14	(12.2)	0.08 (0.01-0.69)	0.02
No	137	(94.5)	101	(87.8)	1	
Cat contact						
Yes	2	(1.4)	3	(2.6)	0.69 (0.3-144.5)	0.2
No	143	(98.6)	112	(97.4)	1	
Soil contact						
Yes	20	(13.8)	14	(12.2)	0.5 (0.1-2.2)	0.3
No	125	(86.2)	101	(87.8)	1	
Eating unwashed vegetables						
Yes	11	(7.6)	6	(5.2)	2.9 (0.5-16.3)	0.2
No	134	(92.4)	109	(94.8)	1	
Parents education						
Illiterate	2	(1.4)	2	(1.7)	0.95 (0.07-13.3)	
Primary school	9	(6.2)	23	(20.0)	0.05 (0.01-0.3)	
High school	69	(47.6)	68	(59.1)	0.4 (1.9-0.9)	
College and above	65	(44.8)	22	(19.1)	1	
Mother's occupation						
Housewife	119	(82.1)	107	(93.0)	0.6 (0.3-1.2)	0.2
Government employment	26	(17.9)	8	(7.0)	1	
Father's occupation						
Government employment	143	(98.6)	105	(91.3)	0.08 (0.01-1.4)	0.08
Farmer	2	(1.4)	10	(8.7)	1	
Water source						
Treated	79	(54.5)	103	(89.6)	1	
Untreated	66	(45.5)	12	(10.4)	20.1 (6.2-65.3)	<0.001
Family atopic history						
Yes	85	(58.6)	5	(4.3)	42.5 (13.0-138.6)	<0.001
No	60	(41.4)	110	(95.7)	1	
***Toxocara* infection**						
Yes	6	(4.1)	1	(0.9)	52.7 (1.7-1578.5)	0.02
No	139	(95.9)	114	(99.1)	1	

analysis. In new analyses, the multivariate analyses identified that older children (age more than 12 years) had a non-significant lower risk to be asthmatic (OR, 0.77; 95% CI, 0.11–5.1; *P*-value= 0.78), although living in rural areas (OR, 0.37; 95% CI, 0.14–0.97; *P*-value= 0.04), contact with dog (OR, 0.08; 95% CI, 0.01–0.69; *P*-value= 0.02), and having parents with higher levels of education (OR, 0.4; 95% CI, 0.16–0.99; *P*-value= 0.04) were significant protective factors for childhood asthma. In comparison with healthy children, case-patients were more likely to have an atopic history in the family (OR, 42.5; 95% CI, 13.0–138.6; *P*-value <0.001), and usage of untreated water (OR, 20.1; 95% CI, 6.2–65.3). No significant association was found between asthma status and contact with cats. The unadjusted and adjusted ORs for the association between asthma and *Toxocara* infection were (OR, 4.9; 95% CI, 0.58–41.4) and (OR, 52.7; 95% CI, 1.7– 1578.5), respectively, suggesting a significant positive association (*P*-value <0.001). Mean total immunoglobulin E (IgE) was significantly higher in *Toxocara*-infected children (222.3 ± 367.1) than in non-infected children (143.19 ± 218.05) in the case group (*P*-value < 0.05). Moreover, *Toxocara* infection was non-significantly more prevalent (OR, 3.79; 95% CI, 0.73–19.7; *P*-value = 0.12) in children with moderate sustainable asthma (9.3%; 3/32) than in children with mild sustainable asthma (2.3%, 3/113).

Table 2 gives the unadjusted and adjusted ORs for potential risk factors of *Toxocara* infection. According to univariate analyses, children who were seropositive for *Toxocara* were more likely to have contact with dogs (OR, 34.7; 95% CI, 6.2–192.2), cats (OR, 10.7; 95% CI, 1.0–107.2), and soil (OR, 123.5; 95% CI, 6.8–2222.8) and also more likely to consume unwashed vegetables (OR, 6.3; 95% CI, 1.1–35.4). However, contact with dog and soil were only potential risk factors for *Toxocara* infection in the multivariate analysis (Table 2).

Discussion

We performed this case-control study to clarify whether there is an association between *Toxocara* infection and childhood asthma. Our main reason for performing this study was the fact that both *Toxocara* infection and asthma are widely distributed in northern Iran and some previous experimental and epidemiological studies have suggested that *Toxocara* infection can play an etiological role in the development of asthma and other allergic disorders. The present epidemiologic study provides support for the association between *Toxocara* infection and childhood asthma, and the association was strong after controlling for confounders. Moreover, our results showed more severe asthma and more total IgE level in asthmatic children with *Toxocara* infection compared with non-infected children.

To the best of our knowledge, the role of *Toxocara* infection in the development of allergic disorders is controversial. The results of our multivariate analysis, when other confounders were adjusted, showed a strong association between *Toxocara* infection and childhood asthma. In agreement with our results, some previous epidemiologic studies showed a significant association related to *Toxocara* infection and childhood asthma,[24-30] although others showed a non-significant association.[31-37] An explanation for these different results could be a difference in the age of the study population, study design, different ethnic populations, genetic, geographical area, and different sensitivity and specificity of diagnostic methods (in-house or commercial ELISA and Western blot). Moreover, a recent meta-analysis indicated a significant positive association between exposure to *Toxocara* infection and increased risk of childhood asthma (OR, 1.91; 95% CI, 1.47–52.47). This association was also persistent in sub-group analysis for both case-control (OR, 2.13; 95% CI, 1.43–3.15) and cross-sectional (OR, 1.73; 95% CI, 1.23–2.44) studies.

Although the underlying pathomechanism is not well established, it seems that somatic migration of *Toxocara* larvae to different organs including lung and intensive human immunological responses to these larvae are the main effectors to the development of asthma.[8,10] In line with this statement, some experimental studies indicated persistent airway hyperresponsiveness, airway inflammation, and diminished lung function in mice following infection with *Toxocara* larvae.[16,38] It has been shown that pulmonary inflammation occurs 48-h post-infection and can continue for up to 3 months.[38] Other possible mechanisms are an increase in specific IgE against *Toxocara* antigens that can bind to high-affinity IgE receptors (FcεRI) on mast cells and lead to degranulation of mast cells and the release of vasoactive substances like histamine.[8,39] In line with this statement, our results showed levels of total IgE in *Toxocara*-infected children which were significantly higher than in non-infected children. Moreover, Pinelli et al.,[16] in an experimental study, indicated that *Toxocara* infection elevated levels of IgE antibody and eosinophil counts in bronchoalveolar lavage fluid, and also the expression of IL-4 mRNA in lung tissue of infected mice.

With respect to risk factors for *Toxocara* infection, we have found that contact with dogs, cats and soil, and also eating unwashed vegetables were potential risk factors in univariate and multivariate analyses. This is in agreement with the results from our previous study among the general population in northern Iran.[20] Moreover, a recent comprehensive meta-analysis evaluating the global prevalence of *Toxocara* infection showed that male gender, living in a rural area, young age, close contact with dogs, cats or soil, consumption of raw meat, and the drinking of untreated water were significant potential risk factors for the acquisition of *Toxocara* infection.[40]

This study has some limitations and the results presented here should be interpreted with regard to these limitations. The major limitation of this study is our low sample size with a low number of seropositive children in both the case and control groups that caused some problems in our statistical analysis. For example, although we used the PLR method to resolve this limitation, large OR and 95% CIs were some of the observed variables. Another limitation was that we were unable to perform Western blot examination on sera samples and also there are no supporting data on blood eosinophils or complete blood count (CBC). The final limitation is related to cross-reaction of IgG-ELISA kit with other helminth infections such as ascariasis, trichinellosis, filariasis, etc. Also, according to

Table 2 Univariate and multivariate analyses of risk factors associated with *Toxocara* infection among children in northern Iran.

Variable	All children (n=260)		Univariate OR (95% CI)	Multivariate OR (95% CI)
	N	%		
Sex				
Male	145	4 (2.8)	1	1
Female	115	3 (2.6)	0.94 (0.21-4.31)	2.08 (0.10-273.31)
Age				
≤6	132	5 (3.8)	1	1
7-12	118	2 (2.7)	0.44 (0.08-2.30)	0.01(0.00-0.72)
13-18	10	0.0	1.10 (0.06-21.36)	0.06(0.00-28.49)
Residence				
Rural	101	2 (2.0)	0.62 (0.12-3.27)	0.01 (0.00-1.22)
Urban	159	5 (3.1)	1	1
Family income				
<1,500,000 T	170	6 (3.5)	3.26 (0.39-27.47)	1.26(0.01-2770.24)
≥1,500,000 T	90	1 (1.1)	1	1
Dog contact				
Yes	22	5 (22.7)	34.71 (6.26-192.29)*	41.61 (2.54-26470.70)*
No	238	2 (0.8)	1	1
Cat contact				
Yes	5	1 (20.0)	10.37 (1.00-107.29)*	3.07 (0.01-1333.67)
No	255	6 (2.4)	1	1
Soil contact				
Yes	34	7 (20.6)	123.55 (6.87-2222.86)*	297.47 (4.92-16069861)*
No	126	0 (0.0)	1	1
Eating unwashed vegetables				
Yes	17	2 (11.8)	6.35 (1.14-35.47)*	1.33 (0.01-91.96)
No	243	5 (2.1)	1	1
Parents education				
Illiterate	4	0 (0.0)	2.68 (0.12-60.22)	5.14 (0.02-8777.06)
Primary school	32	1 (3.1)	0.90 (0.09-9.01)	0.16 (0.00-103.64)
High school	137	3 (2.2)	0.63 (0.12-3.18)	1.31 (0.03-584.26)
College and above	87	3 (3.4)	1	1
Water source				
Untreated	78	1 (1.3)	2.63 (0.31-22.17)	1.91(0.05-66.60)
Treated	182	6 (3.3)	1	1

*Statistically significant (*P*-value <0.01).

our experience in previous studies, the NovaTec kit has not shown cross-reactivity with ascariasis.[20,22]

In conclusion and notwithstanding the abovementioned limitations, the results of the present study provide strong evidence that *Toxocara* infection may be associated with childhood asthma. More longitudinal epidemiologic and experimental studies are needed to further explore the role of *Toxocara* infection in the development of childhood asthma and also to elucidate the immunological and molecular mechanisms that underpin this association. Furthermore, according to our findings about risk factors of *Toxocara* infection in the studied area, personal health education and applying the preventive measures to avoid exposure to *Toxocara* infection in children seems necessary in the studied area.

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Conflict of interest

None of the authors have any conflict of interest.

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