Int J Enteric Pathog. 2014 July; 2(3): e15908. DOI: 10.17795/ijep15908

Published online 2014 July 20. **Research Article**

Comparison of Toxocariasis Frequency in Hyper- eosinophilic and Non-Eosinophilic Individuals Referred to Abadan Health Centers

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Received: November 1, 2013; **Revised:** April 16, 2014; **Accepted:** April 25, 2014

Background: Toxocariasis is a zoonotic helminthic infection of humans and animals caused by the larvae of intestinal parasites of dogs and cats (*Toxocara canis* and *Toxocara cati,* respectively). These nematodes develop in to their adult stage in the intestines of cats and dogs. Three clinical entities have been recognized in humans; visceral larva migrans, ocular larva migrans and covert toxocariasis. Eosinophilia is a common finding in infected patients

Objectives: In this study the frequency of toxocariasis in eosinophilic and non-eosinophilic individuals referred to the laboratory of Abadan health centers was compared.

Materials and Methods: Serum samples were collected from individuals attending the laboratory of health centers for any medical problem and were tested for complet blood count (CBC). The samples of patients were divided in to two groups, those with more than 10% peripheral eosinophils, as the eosinophilic group (n = 54) and those with normal eosinophils (0-3%) as the non-eosinophilic group (n = 54). Samples were examined for anti-oxocara IgG by the enzyme linked immunosorbent assay (ELISA) and confirmed western blotting.

Results: Anti-oxocara IgG was detected in the sera of six (11.11%) cases from the eosinophilic group and two (3.7%) of the non-eosinophilic group by the ELISA method, but all had negative results for the western blot analysis.

Conclusions: The results of this study indicated that the eosinophilic individuals might beexposed to other helminthic infections or allergic agents. Further studies are required with more samples with different ages and occupations.

*Keywords:*Toxocariasis; Eosinophilic; *Toxocara Canis*; *Toxocara Cati*

1. Background

Toxocariasis has a worldwide distribution and its prevalence can vary in different parts of the world depending on climatic zones and is generally associated with low socioeconomic level (1). The disease is a zoonosis helminthic infection of humans and animals, caused by the larvae of *Toxocara canis* and *Toxocara cati*. These nematodes are enteric parasites and develop in to their adult stage in the intestine of cats and dogs via the ingestion of eggs containing the second stage larvae; in infected pregnant females, larvae can penetrate through the placenta to infect the fetus. Young puppies or kittens may also be infected with larvae through the milk or by ingesting eggs (2). *Toxocara canis* is regarded as the main cause of human toxocariasis. Humans become infected by ingesting embryonated Toxocara eggs from soil, dirty hands, geophagia and vegetables (3). After the eggs are swallowed, larvae hatch in the intestine wall and are spread through the blood stream. This is termed visceral larva migrans or toxocariasis. The larvae can enter the liver, lungs, brain, eyes and other organs (4). Three clinical entities have been recognized in humans including visceral larva migrans (VLM), ocular larva migrans (OLM) and covert toxocariasis (CT). Eosinophilia is a common finding in infected patients. Visceral larva migrans is characterized mainly by fever, hepatomegaly, splenomegaly, respiratory disorders, hyper- gammaglobulinemia, cough, abdominal pain, hepatomegaly, skin lesions and eosinophilia (5). On the other hand, OLM is caused by larvae invasion of the eyes and their pathological effects include leucocoria, chorioretinitis, optic papillitis, endophthalmitis, and can lead to a partial or complete loss of vision (6). Toxocariasis is one of the causes of eosinophilic infiltration of internal organs (7). The rate of infection variesfrom 1% in Spain (8) to 25.6% in Shiraz (9) and 86% in St. Lucia (1). Toxocariasis is diagnosed using serological tests such as enzyme linked immunosorbent assay (ELISA). The use of excretory-secretory antigens from the second-stage larvae of *T. canis* increases the specificity and sensitivity of

the ELISA (10-12). The use of monoclonal antibodies has proved effective for the diagnosis of active cases (13).

2. Objectives

The aim of this study was to assess the IgG type antibody specific to Toxocara species in groups of eosinophilic and non-eosinophilic individuals referred to the laboratories of Abadan health centers.

3. Materials and Methods

The study was carried out from June 2011 to June 2012 in Abadan city. The city is 100 kilometers from Ahvaz city, the capital of Khuzestan province, southwest of Iran. Blood samples were collected, from individuals referred to the laboratory of health centers, for medical problems and stored at -20°C. Hyper- eosinophilia was defined as a level equal to 10% or more than 10% (> 500 eosinophil/μL) of the complet blood count (CBC) consisting eosinophilic group. In total, 54 eosinophilic individuals were selected, including 25 males and 29 females. The control group (non-eosinophilic) consisted of 54 individuals including 25 males and 29 females with normal peripheral eosinophil count. Sera were separated by blood centrifugation at 3000 rpm for 5 minutes and kept in -20 °C until use. Each individual completed a questionnaire. The mean age of each group was 39 years (Tables 1 and 2). Specific IgG antibody against Toxocara ES antigens was measured using the Toxocara ELISA kit (IBL, Germany) and the western blot technique (LDBIO, France) was used for confirmation.

4. Results

According to the ELISA method, frequency of anti-*Toxocara* antibody (Ig G) in eosinophilic individuals was six (four females and two males) (11.11%) (Table 3), whereas in non-eosinophilic individuals this frequency was two (one female and one male) (3.7%) (Table 4). The rate of infection was significantly higher in females. Table 5 shows the mean blood factors in positive eosinophilic individuals. Toxocariasis in none of the positive cases was confirmed by the western blot method.

Table 1. Frequency of Hyper- osinophilic Individuals According to Age and Gender

Gender	Age, y					
	10- 20	21- 30	31- 40	41- 50	> 50	Total
Male	4	5	9	7	0	25
Female	6	7	5	7	4	29
Total	10	12	14	14	4	54

Table 2. Frequency of Non- Eosinophilic Individuals According to Age and Gender

Gender	Age, y					
	10- 20	21- 30	31- 40	41- 50	> 50	Total
Male	1	4	9	7	4	25
Female	4	6	7	7	5	29
Total	5	10	16	14	9	54

Table 3. Frequency of Toxocariasis in Eosinophilic Individuals According to Gender

Result	Gender		
	Male	Female	Total
Negative	23	25	48
Positive	2	4	6
Total	25	29	54

Table 4. Frequency of Toxocariasis in Non-Eosinophilic Individuals According to Gender

Result	Gender		
	Male	Female	Total
Negative	28	24	52
Positive	1	1	2
Total	29	25	54

Table 5. Hematological Factors in Eosinophilic Individuals

Factor	Minimum	Maximum	Mean ± SD	Normal Range
Hemoglobin	11.2	13.4	12.3 ± 1.1	11 - 16 g/dL
Hematocrit	34	39.3	36.65 ± 2.65	37 - 50%
RBC	5.8	12	8.4 ± 3.14	$4\text{-}10 \times 10^{6}$ μL
WBC	4.08	4.58	4.33 ± 0.35	$3.5\text{-}5.5 \times 10^{3}$ μL
Neutrophil	38	58	48 ± 10	50 - 70%
Lymphocyte	20	38	29 ± 9	20 - 40%
Eosinophil	18	31	24.5 ± 6.5	0 - 3%
Monocyte	1	2	1.5 ± 0.5	0 - 4%
Basophil	0	1	0.5 ± 0.5	0 - 0.5%
Platelets	158	321	239.5 ± 81.5	$150\text{-}450 \times 10^{3}$ μL

5. Discussion

Toxocariasis has a worldwide distribution. Its prevalence can vary in different parts of the world depending on climatic zones (14); 26.8% in Brazil (15) and 2.5% in Danmark, (16). In a Turkish study, antibodies specific to Toxocara were detected in 32.6% of eosinophilic patients and 20.3% of the non-eosinophilic group (17). In the present study 11.11% of the eosinophilic group and 3.7% of the non-eosinophilic group had anti-Toxocara IgG antibody. All positive cases detected by the ELISA method had negative results when examined by the western blot technique. This studysuggests that despite the relatively low level of exposure to toxocariasis among the studied population, antibodies specific to Toxocara were detected in 19% of hypereosinophilic patients (18) and 2% in school children of Ahvaz city and its suburbs (19). Demographic and socioeconomical factors may lead to an increase in toxocariasis. The lower sero-prevalence of toxocariasis (11.11%) in the present study compared to other previous studies may be due to factors such as age, culture, feeding pattern and geographical region. Age is one of the factors affecting the results. There are different results presented by researchers on the relationship between the frequency of Toxocara and age. Some of studies found no significant change with age, whereas others claimed that Toxocara is more frequent in children (20). Among all age groups, children are most vulnerable and prone to infection mainly because of their frequent contact with animals (dogs and cats) and contaminated soil. In our study, allindividuals were above 10 years of age and there was no significant difference in the rate of infection related to age (> 0.05). A high prevalence of infection among adults probably indicates past infection by *Toxocara* agents, because larvae can survive inside the body for 10 years (21). In the present study there was a significant difference in the frequency of infection with gender, where the frequency was higher in females (< 0.05). This finding resembled the results of the study by Mangwal and Bixton (1993) (22) and may be due to working in the kitchen and being exposed to contaminated vegetables and fruits and other infected sources.

It has been determined that toxocariasis can be transmitted orally by consuming infective eggs, which are found in soil contaminated with feces of infected cats and dogs. Thus, individuals at greater contact with soil contaminated with cat and dog feces have higher antibody detection rates (4). In the present studythe subjects probably had no contact with animals or soil. Although the frequency of owners of dogs in rural areas of Iran is considerable yet, due to Islamic beliefs close contact of humans with dogs are unremarkable and consequently people living in these areas are not easily infected with *T. canis*.

Abadan city is considered to be an endemic area for intestinal parasites, which may have cross reactivity with Toxocara. Although in our study the highest seropositivity was also found for eosinophilic individuals, yet this was not statistically significant. It has been suggested that helminthic infections, malignancy and allergic diseases can cause an increase in eosinophil counts in peripheral blood. It has been accepted that the most common eosinophilia cause worldwide is parasitic infection. It is well known that in making a diagnosis based on the antigen-antibody reaction, nonspecific reactions and the small sample size might have affected our study results.

This study provides basic information on the presence of toxocariasis in our region and the results of this study indicate that eosinophilic individuals might be exposed to other helminthic infections or allergic elements. Further studies are required with more samples with different ages and occupations.

Acknowledgements

This study was part of the MSc thesis (B- 90/0011) of Mrs Fatemeh Shamakhteh conducted under the financial support of Ahvaz Jundishapur University of Medical Sciences (AUJMS). We would like to express our appreciation to the Vice Chancellor of Research Affairs, Thalassemia and Hemoglobinopathy Research Center and Abadan Arvand International Division, Ahvaz Jundishapur University of Medical Sciences, Dr. Gholamabbas Kaydani and Mr. S. Rashtipour for their critical help.

Funding/Support

Vice Chancellor of Development, Research and Technology of Ahvaz Jundishapur University of Medical Sciences and Abadan Arvand International Division provided Funding and support.

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