

Human toxocariasis seroprevalence among patients with uveitis in Alborz Province, Iran

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Abstract

Introduction and objective. Toxocariasis, predominantly caused by *Toxocara canis*, is a common zoonotic parasitosis worldwide. *Toxocara* infection is a cause of vision impairment and blindness. The presented study investigates the frequency of antibodies against *Toxocara* among uveitis patients and the epidemiological factors associated with disease.

Materials and method. Fifty-four patients with uveitis and 59 healthy subjects were studied. Anti-*Toxocara* antibodies status was determined in all serum samples using enzyme linked immunosorbent assay (ELISA), and seropositive samples analyzed by Western blot (WB) technique.

Results. The frequency of *Toxocara canis* infection was found to be significantly higher in uveitis patients, compared to healthy controls by the use of ELISA test, being 14.8% and 1.7%, respectively. From 8 seropositive samples, 5 (62.5%) patients exhibited *Toxocara* immunoglobulin G (IgG) antibodies in response to Western blot, whereas in the control group, none were detected positive by Western blot. No significant difference was found between pet owners, nor between different places of residence. The seroprevalence to *Toxocara* among uveitis patients was significantly related to gender, age and medical diagnosis. The highest prevalence was found in patients with posterior uveitis (27.8%).

Conclusion. Anti-*Toxocara* antibody titers are associated with the risk of vision impairment -uveitis. The risk factor associated with *Toxocara* exposure identified in this study warrants further investigation.

Key words

toxocariasis, diagnosis, uveitis patients, *Toxocara canis*, ELISA, Western blot

INTRODUCTION

Toxocariasis is a worldwide zoonotic parasitic disease caused by the larval stage of either *Toxocara canis* (dog ascarid) or *Toxocara cati* (cat ascarid) [1]. Humans become infected with *Toxocara* species when they unintentionally ingest embryonated eggs that have been shed in the faeces of infected animals, or larvae through undercooked paratenic hosts [2, 3]. After ingestion of the eggs, infective larvae are released in the small intestine, and these subsequently penetrate the intestinal wall, enter the circulation, and migrate to different organs: the liver, lungs, brain, and eyes, where they induce inflammatory reactions and symptoms [4]. Based on the infected organs, it is clinically classified into visceral larva migrans (VLM), ocular larva migrans (OLM), neurotoxocariasis (NT), and covert toxocariasis (CT) [5, 6].

Toxocara larvae that migrate to the eye are trapped, resulting in an eosinophilic inflammatory mass. The syndrome takes several weeks to become clinically apparent, but the patients present with unilateral visual deficits, ocular pain, leukocoria, or strabismus. The retinal lesion

most commonly presents with chronic unilateral uveitis, and a marked vitreous opacification that overlies a primary eosinophilic granuloma [6, 7]. Posterior and peripheral retinochoroiditis, as well as optic papillitis, have also been reported. The inflammatory response created by ocular involvement may result in epiretinal membrane formation and traction retinal detachment [8]. Ocular toxocariasis is a clinically well-defined manifestation of intraocular infection by *Toxocara* larvae. The age at presentation in patients with ocular toxocariasis may vary from 1–77 years of age. Its clinical manifestation can be classified as posterior pole granuloma, peripheral granuloma, nematoda endophthalmitis, and atypical presentation [9, 10]. In Japan, an epidemiologic study showed that ocular toxocariasis was recorded for 1.1% of all uveitis patients [11]. In a survey from the Republic of Korea, a high seroprevalence of 38.8% (19/50) intermediate uveitis was reported by enzyme-linked immunosorbent assay in a Suwon population [12].

In Iran, *Toxocara* infection has been reported in most areas. The seroprevalence rate of human toxocariasis varies between 2.7–29.3%. Reports from several regions also provide documented evidence of an increase in definitive hosts in recent years. The seroepidemiology of animal toxocariasis varies in different geographical regions, ranging from 3.3 to higher than 78.8% in different areas [13]. In a study in

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Karaj, an urban area in Iran, the prevalence of *Toxocara* spp eggs was 36.4% in public parks [14]. Therefore, the current study aimed to assess the seroprevalence of toxocariasis in ocular manifestation and the epidemiological factors associated with the disease in patients who are considered to have uveitis.

MATERIALS AND METHOD

Ethical clearance. The purpose and procedures of the study were explained to all participants, and a written informed consent was obtained from all of them. The study was approved by the ethical clearance committees of the Alborz University of Medical Sciences (No. ABZUMS.REC.1395, 103).

Study area. Karaj, in the centre of Alborz Province, is located in the north of Iran, bordering with the states of Tehran, Qazvin, Markazi, and Mazandaran. In 2011, the estimated population of Karaj was 2,412,513, of which 90.5% lived in urban areas. The study area (35° 48' 57" N, 50° 58' 29" E) comprised the largest city in Alborz Province. The areas – Karaj city and Alborz Province – are situated within a cold, semi-arid climatic zone, and receives most rainfall (annually 260 mm) between March – May; mean annual temperature – 15.1 °C (https://en.wikipedia.org/wiki/Alborz_Province).

Subjects. This study was conducted on 54 uveitis patients (21 women and 33 men) of the 272 individuals with intraocular inflammation of unknown etiology (various combinations of blurred or reduced vision, visual floaters, eye discomfort or pain, and intolerance to light), who were evaluated regarding their medical history, clinical characteristic at the Division of Ophthalmology, Educational and Therapeutic Centres or hospitals related to Alborz University of Medical sciences from April – December 2016. Inclusion criteria were inflammation of the uveal tract (iris, ciliary body, and choroid) diagnosed as anterior, intermediate, posterior uveitis, and idiopathic uveitis. Demographic characteristics of participants included age, gender, place of residence, keeping pets, history of diseases, and eye involvements, recorded during sample collection.

Control population. The control group consisted of 24 male and 35 female volunteers aged between 7–69 years with comparable epidemiology characteristics and without any complains or history of previous ocular complications in either themselves or their families. The demographic and lifestyle characteristics were obtained by study questionnaire.

Sample collection. Peripheral venous blood samples were collected during the same visit in which the patients and healthy volunteers were clinically examined to perform a haemogram with leukocyte differentiation, and measurement the red blood cells (RBCs) count. Eosinophilia was considered as an absolute eosinophil count of more than 350/mm³. Sera from participants were kept frozen at -20°C until serological analysis.

Light microscopy. The blood smears were air-dried, fixed in methanol, stained with 3% Giemsa solution, and diluted in phosphate buffer saline (pH=7.2) for 20 min. The blood films were examined under a microscope with an oil immersion

lens at a 1,000× power field. Haematological data, such as eosinophil levels, were recorded.

Toxocara-ELISA screening test. Anti-*Toxocara canis* antibodies (IgG) were detected by commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (IBL, International GmbH, Hamburg, Germany), following the manufacturer's guidance. In brief, diluted sera samples (1:100) were added to wells; after 30 min incubation at room temperature, horseradish peroxidase-conjugated goat anti-human IgG was added at a 1:1000 dilution (30 min at room temperature), followed by the tetramethylbenzidine (TMB) substrate. Absorbance readings were made at 620 nm; cut-off absorbance value was defined as the mean absorbance reading for 3 negative control sera plus 2 standard deviations. Compared to negative and positive controls, samples with absorbance ≤ 9 were considered negative while absorbance ≥ 11 was considered positive, between 9.1–10.9 as equivocal. Antibodies levels were expressed as reactivity indices, which were calculated as the cut-off value; positive samples had reactivity indices of more than 0.150.

Toxocara-Western Blot analysis. Western Blot (WB) of the positive samples was performed and results interpreted according to the manufacturer's instructions, using WB IgG commercial kit (LDBIO Diagnostic, Lyon, France). Briefly, the strips were incubated for 120 min at 37°C, with sera at a dilution of 1:100. Before being washed at least 3 times in PBS containing 0.1% Tween 20, the strips were further incubated for 120 min at 37°C with the second antibody, anti-human IgG peroxidase conjugate (dilution 1:1,000). After washing, as mentioned previously, the substrate diaminobenzidine (DAB) was added and the reaction stopped by several washes in distilled water. The results were considered as positive when the samples reacted to 2 or more low-molecular-weight bands (LMWB 30 – 45 kDa).

Data analysis. Statistical evaluations were performed using SPSS software version 15.0. All epidemiological clinical and laboratory information were tested for association with *Toxocara* infection. Chi-squared and Fisher exact tests were used for categorical data. A *P*-value less than 0.05 was considered statistically significant.

RESULTS

IgG antibody positivity by ELISA with relation to demographic characteristics. Based on the evaluation by ELISA, of the 54 patients who participated in the study, 8/54 (14.8%) had positive anti-*Toxocara canis* antibodies, compared to 1/59 (1.7%) among the healthy group. The overall seroprevalence in the group of patients showed a significant dependence on gender and age ($P < 0.05$) – 6.1% (2/33) in men and 28.6% (6/21) in women. The seroprevalence was highest (28.6%, 4/14) in group 2 (10–19 years), followed by 14.3% (1/6) in group 5 (≥40 years) (Tab. 1). Uveitis patients who lived in rural areas demonstrated a higher seropositivity to *Toxocara* (17.6%), compared to those in urban areas (13.5%), but the difference did not attain a significance level ($P > 0.05$). Risk factors analysis indicated that the seropositive rates among the studied patients who kept pets or did not keep pets were 20.0% (4/20) and 11.8% (4/34), respectively ($P > 0.05$).

Table 1. Seroprevalence to *Toxocara canis* determined by ELISA test in uveitis patients and control group with relation to demographical characteristics

| Factors | Uveitis patients, N (%) | Control group, N (%) | Statistical analysis (P-value)† |
|----------------------------------|-------------------------|----------------------|---------------------------------|
| Age group (years) | | | |
| 0–9 | 0/2 (0.0) | 0/4 (0.0) | < 0.05 |
| 10–19 | 4/14 (28.6) | 0/14 (0.0) | |
| 20–29 | 0/4 (0.0) | 1/5 (20.0) | |
| 30–39 | 3/27 (11.1) | 0/30 (0.0) | |
| ≥40 | 1/7 (14.3) | 0/6 (0.0) | |
| Gender | | | |
| Male | 2/33 (6.1) | 0/24 (0.0) | < 0.05 |
| Female | 6/21 (28.6) | 1/35 (2.9) | |
| Pet owner (cats and dogs) | | | |
| Yes | 4/20 (20.0) | 0/15 (0.0) | Not significant |
| No | 4/34 (11.8) | 1/44 (2.3) | |
| Residency | | | |
| Rural | 3/17 (17.6) | 0/20 (0.0) | Not significant |
| Urban | 5/37 (13.5) | 1/39 (2.6) | |
| Eosinophilia | | | |
| Yes | 2/5 (40.0) | 1/11 (9.1) | < 0.05 |
| No | 6/49 (12.2) | 0/48 (0.0) | |

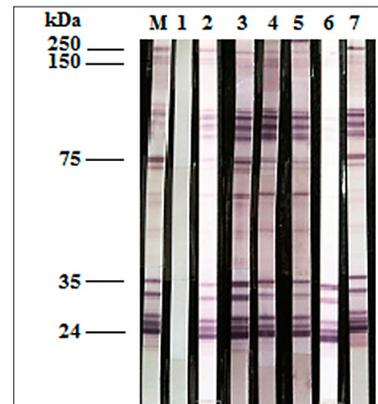
† Significance by the χ^2 test**Table 2.** Seroprevalence of antibodies to *Toxocara canis* in the uveitis cases considering medical diagnosis

| Uveitis | Patients, N (%) | Statistical analysis |
|--------------|-----------------|----------------------|
| Anterior | 1/6 (16.7) | < 0.05 |
| Intermediate | 1/18 (5.6) | |
| Posterior | 5/18 (27.8) | |
| Idiopathic | 1/12 (8.3) | |

Regarding medical records, there was a predominance of posterior, uveitis compared to other forms of uveitis among the seropositive patients (Tab. 2). Of 8 seropositive patients, 27.8% (5/18) were associated with posterior, 16.7% (1/6) with anterior, 5.6% (1/18) with intermediate and 8.3% (1/12) with the idiopathic form of uveitis (Tab. 2). The dependence of the seropositivity to *Toxocara canis* on the clinical form of uveitis was statistically significant ($P < 0.05$).

IgG antibody positivity by ELISA with relation to microscopic blood analysis. Five of the 54 patients with uveitis (9.3%) and 11 of the 59 healthy people (18.6%) showed eosinophilia, with a range between 360 – 980/mm³. Among 5 patients with uveitis and eosinophilia, 2 (40.0%) showed the presence of anti-*Toxocara* IgG antibodies, while among 11 healthy people with eosinophilia, there was only one person (9.1%). The difference proved to be statistically significant ($P < 0.05$) (Tab. 1). This result suggests an association between the uveitis caused by *Toxocara* and eosinophilia.

Western blot. Of 8 seropositive samples by ELISA, 5 (62.5%) tested positive for IgG antibodies against the excretory-secretory larval antigens of *Toxocara canis* by Western blot (TcES-WB). The positive WB strips showed at least 2 or more bands of lower molecular weights (Fig. 1).

**Figure 1.** Western blot results for sera of the studied population whose IgG antibodies were reactive to low-molecular-weight bands at 30 and 35 kDa, which are specifically related to *Toxocara* infection. Lane M: protein molecular weight marker; lane 1: toxocariasis-negative serum; lane 2: toxocariasis-positive serum; lanes 3–7: sera of patients with *Toxocara canis* (ELISA-positive reacted).

DISCUSSION

Human infection by *Toxocara* spp. may result in visceral and ocular larva migrans with various types of morbidity and has thus drawn considerable attention in recent years [15]. Toxocariasis has a worldwide geographical distribution and the seropositivity of *Toxocara* antibodies varies from 2.4% – 76.6% [16–18]. Although human toxocariasis is one of the most common zoonotic infections worldwide, there are only a few studies that estimate the frequency of ocular larva migrans. The annual incidence of ocular toxocariasis in different parts of the world ranges from 17–52 per 100,000 persons, and the prevalence ranges from 58–115 per 100,000 people [9]. Ocular toxocariasis can induce inflammation of the uveal and retinal tissues, which has been revealed as uveitis, and it can result in diverse lesions, from a simple peripheral pigmentary retinal scar to macular damage that cause severe visual loss after treatment [9].

In this study, the frequency of *Toxocara* infection in uveitis patients was 14.8%, significantly higher than in healthy controls (1.7%). However, as the reported prevalence of toxocariasis in the general population in the area examined ranged from 2–25.6%, the relatively low frequency of *Toxocara* infection in the control group required cautious interpretation. Using excretory-secretory antigens of *Toxocara canis* larvae by ELISA (TcES-ELISA), a same in the current study, association studies between toxocariasis and uveitis in a Korean population revealed anti-*Toxocara* antibodies (IgG) in 38.0% of the 50 participants [12]. Different serological studies have been undertaken regarding the seroprevalence of toxocariasis in patients with ocular inflammatory disorder in different geographical regions of the world [19, 20]. The rate

of seroprevalence of toxocariasis in the presented findings is very similar to the rate of infection in other parts of the word [21–24].

In most laboratories, the current methods of detecting *T. canis* infection in humans are typically based on TcES-ELISA [12]. Although TcES-ELISA has been reported on yield reasonable levels of specificity (96.7%) and sensitivity (97.0%), the specificity is excessively low to be reliable when assaying communities in which several of intestinal cross reacting helminths are common [19]. Thus Western blot, rather than a general ELISA, was employed in the current survey. Immunoblotting results of 8 ELISA seropositive samples in the patients and one case in healthy control in the present study showed that 62.5 (5/8) was positive by TcES-WB. Limited studies are available in seroprevalence of toxocariasis in patients with uveitis. In an investigation, it has been shown that the seroprevalence of toxocariasis in uveitis patients using *Toxocara* excretory secretory-WB (TES-WB) was 2.6% [20].

Results of the presented study suggest a high (40.0%) seroprevalence of *Toxocara* exposure in patients with eosinophilia. The seroprevalence found in these patients was higher than those (9.1%) reported in the control group ($P < 0.05$). A similar study by Kwon et al. [12], investigated the association between intermediate uveitis and toxocariasis in a Korean population. In the analyses of intermediate uveitis patients with or without ocular toxocariasis, the increasing eosinophils was significantly different between the 2 groups. The seropositivity rate to *Toxocara* infection in the hyper eosinophilia patients with intermediate uveitis were significantly higher than in non-ocular toxocariasis group [12].

According to the literatures, multiple risk factors have been associated with *Toxocara* epidemiology, such as age, gender, geophagia, keeping pets, playing in sand boxes, and climatic condition [25, 26].

In the presented study, seropositive results were obtained in females, a finding that could be explained by the culture of the area where women more than men come into contact with cats and dogs. The findings of the current study are in sharp contrast with those of Einipour et al. [20] and Kwon et al. [12], who reported that toxocariasis predominantly in males.

The major risk population in the study was the age group 10–19 years, significantly associated with seropositivity. Toxocariasis is seen frequently among children and young adults probably due to poor hygiene. The relatively gradual increase in seropositivity rate associated with age suggested that soil exposure, which is greatest during childhood years, may not be the principal mechanism by which people are exposed to *Toxocara* [27]. In the current study, uveitis patients consisted of various age groups, from children to the elderly, with ages ranging between 8–87 years-old. The seropositivity was affected by age, though there were significantly more seropositive uveitis patients who were students. The current findings support a previous observation made by Pak et al. [25].

Contact with cats and dogs might represent a risk for toxocariasis. The presented results indicated that the subjects in both groups who exhibited a history of keeping dogs and cats demonstrated a higher risk of *Toxocara* infection than those who did not; however, these effects were non-significant in the statistical analysis. This finding could be explained by the culture of the area, where persons are less likely to come

into contact with dogs and cats, or keep them in the home.

Another risk factor identified in the presented study was the rural residence, which could be explained by the diet of people in the region, who had consumed encapsulated larvae in the raw or uncooked meat of the paratenic hosts, such as cows, sheep, and chickens.

A main finding of the presented study was the demonstration of a significant relationship between seropositivity to *Toxocara* and the type of inflammation of the uvea. Of the 8 patients classified as having anti-*Toxocara* antibodies, 5 (27.8% of total examined and 62.5% of total positive) had a posterior uveitis. This observation suggested that ocular toxocariasis can induce inflammation of uveal and retinal tissues that results in a posterior uveitis. These findings confirmed the earlier results indicating that posterior uveitis is the most common form of clinical presentation, consisting of 44.0% of ocular toxocariasis cases [28]. A case of ocular toxocariasis with posterior pole granuloma has also been reported [23].

CONCLUSIONS

Taken together, the obtained results corroborate some earlier observations on an association between *Toxocara* infection and uveitis. Thus, the investigation of anti-*Toxocara* IgG using ELISA should be a routine test in the people with retinal granuloma or uveitis, particularly in its posterior form.

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