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Short Communication

Frequency of Toxocara Antibodies in Patients Clinically Suspected to Ocular Toxocariasis, Northeast of Iran

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Abstract

Background: Human toxocariasis is a neglected parasitic disease in most countries including Iran. Among different clinical forms of toxocariasis, ocular toxocariasis (OT) is an important disease resulting in severe vision loss. However, the prevalence and incidence of OT are currently unclear in Iran. This study aimed to determine the prevalence of ocular toxocariasis among patients with uveitis in the Northeast of Iran.

Methods: From 2015 to 2017, 510 patients with uveitis referred to Khatam-al-Anbia, a tertiary eye hospital at Mashhad, Iran were examined for OT. Serum samples of the suspected patients were obtained and evaluated for IgG against *Toxocara canis* using ELISA test. Anti-*Toxocara* IgG positive serums were further investigated using confirmatory Western blotting (WB) analysis.

Results: Twenty patients had pathologic changes and clinical presentations in the anterior and posterior segments of their eyes and they were clinically diagnosed ocular toxocariasis. Among the 20 patients, 2 (10%) patients showed IgG antibody against *Toxocara canis* on ELISA as well as on WB test. The calculated prevalence of ocular toxocariasis was about 0.4%.

Conclusion: Ocular toxocariasis can be diagnosed both clinically and serologically in Mashhad, northeastern Iran. Although OT is a rare pathologic eye disease, it should be considered as one of the important cause of infectious posterior uveitis.

Introduction

Toxocara canis (*T. canis*) and *Toxocara cati* (*T. Cati*) are the parasites of dogs and cats, respectively. Man can be infected by ingestion of fertilized eggs or larvae via direct contact with carnivores or via eating uncooked meat, liver, etc., (1). When the eggs hatch, the larvae penetrate through intestinal mucosa and migrate through blood into different organs. This phenomenon is called visceral larva migrans (VLM) (2), which is more common than ocular larva migrans (OLM). About 80% of patients with OLM are under 16 yr old and mostly seen in boys (3).

Diagnosis of OT is based on the histological demonstration (It is risky and non-routine), clinical manifestations, typical fundus lesions, supportive laboratory tests and ocular imaging findings. Definitive diagnosis of ocular toxocariasis can be difficult especially in OT patients when their serum antibodies are below the cut-off level (4). Current diagnosis of OT is made by the typical ophthalmologic signs along with the positive result of serum antibody (5). The most frequent clinical presentation of OT is unilateral blurred and decreased vision associated with leukocoria, floaters, strabismus, pain and red-eye. Bilateral ocular involvement is very rare (4, 6). In children, unilateral ocular lesions including chronic vitritis, macular and peripheral granuloma or leukocoria are the characteristic clinical signs suggestive of OT.

Serological tests can be used as a suitable screening test for detection of human toxocariasis (7, 8). *Toxocara* larva is responsible for 1% and 0.01% of the patients with uveitis in Asian countries (especially Korea and Japan) and USA, respectively (9, 10). Only a few *Toxocara* larvae cause ocular lesions in human which may lead to permanent blindness (11).

During recent decades, the desire to keep pets especially dogs and cats in home increased. However, epidemiological data of OT in Iran is currently unclear (12). This study aimed to detect anti-*Toxocara* antibody among patients with uveitis referred to Khatam-al-Anbia eye Hospital, Mashhad, Northeastern Iran.

Materials and Methods

This cross-sectional study was performed on patients with uveitis referred to Khatam-al-Anbia Eye Hospital, Mashhad University of Medical Sciences (MUMS), northern of Iran between 2015 and 2017. Medical records of the patients with unknown etiology of anterior and posterior uveitis were obtained. Blood serum samples of 20 patients clinically diagnosed as OT were collected.

The study adhered to the tenets of Helsinki Declaration and was approved by the Ethics Committee at MUMS (Ethical code: IR.MUMS.fm.REC.1394.523).

The obtained serum samples were analysed to measure IgG antibody against *T. canis* using Enzyme Linked Immuno-Sorbent Assay (ELISA) kit (product number: TOCG0450, Dietzenbach, Germany). The kit was known to have high specificity and sensitivity of >95%. ELISA procedure was performed according to the manufacturer's instructions.

The results considered to be valid as the absorbance of the substrate blank was ≤ 0.100. Positive control was ≥10% over the cut-off point. Negative control was ≤0.200. The mean absorbance of cut-off was between 0.150-1.30. Samples with an absorbance value between 10% above or below the cut-off point were not considered as positive or negative (grey zone).

According to the manufacture's recommendations, an index positive more than 11 NTU (NovaTec Unit) was considered positive, and less than 9 NTU as negative for *T. canis* infection. The Western blot test was used to confirm the positive ELISA results (LDBIO Diagnostic, Lyon, France).

Results

Of 510 cases with uveitis, 20 patients were highly suspected of OT based on clinical manifestations. The symptoms and signs of 20 patients are listed in Table 1. Among 20 patients, only 2 (10%) were proven to be anti- *Toxocara* positive patients (Fig.1).

Table 1: Clinical findings of 20 patients highly suspected to ocular toxocariasis

Clinical Findings	***NO.***	***(%)***
Decreased & Blurred vision	20	100
Granuloma	17	85
Vitritis	15	75
Macular edema	7	35
Retinal detachment	4	20
Strabismus	2	10

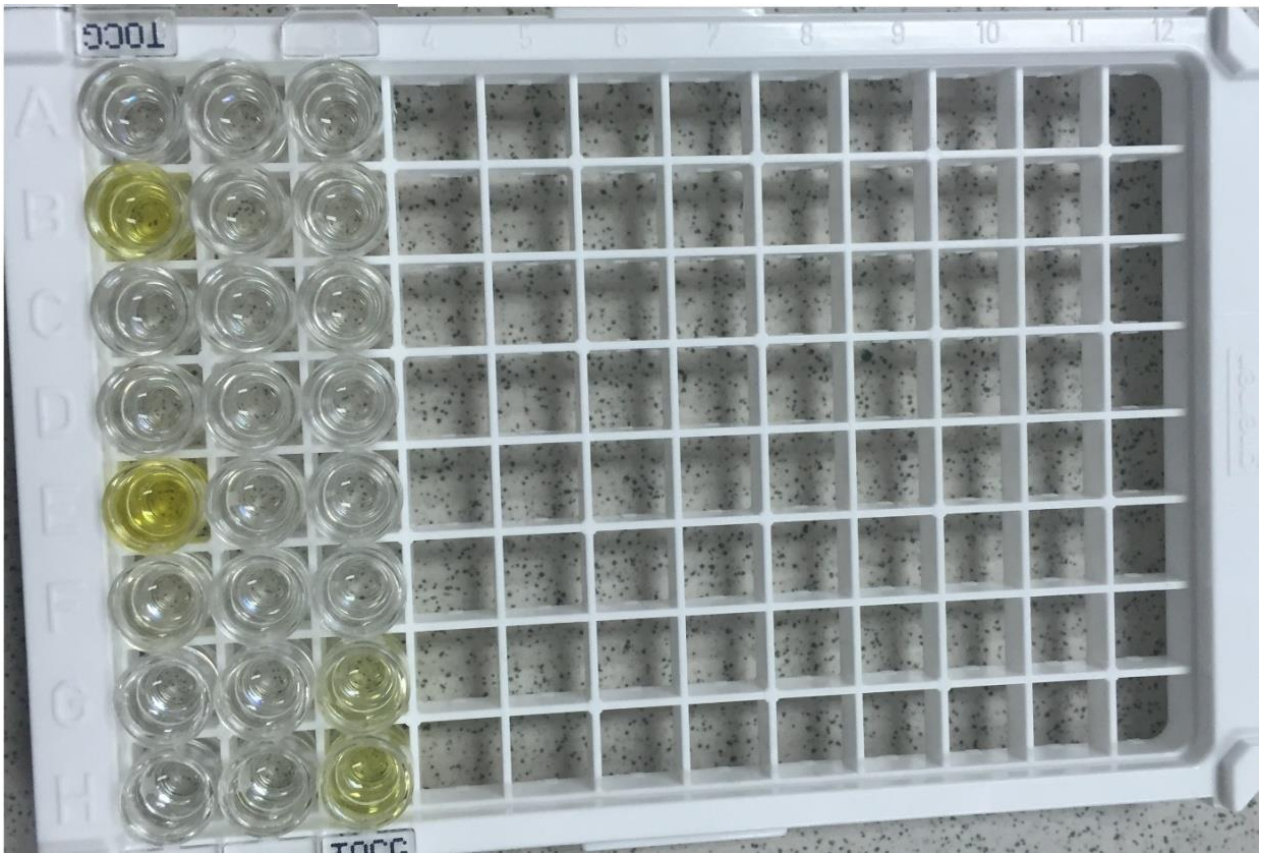

Fig. 1: Two patients (B and E) with ocular *T. canis* IgG antibody in ELISA kit

As mentioned in Table 2, one of the patients was a 45 yr old female from coastal city of Caspian Sea who had history of visual deficit in the right eye since childhood. She presented with decreased visual acuity, posterior uveitis with macular scar in the right eye. She suffered from diabetes but did not have any immunocompromised disorder.

Table 2: Demographic and clinical features of 2 patients with uveitis and anti-*Toxocara canis*

Features	***Patient 1***	***Patient 2***
Gender	Female	Male
Age /y	45	5
Clinical Manifestation	Decreased visual acuity Posterior uveitis Central macular scar	Strabismus Vitritis (Intermediate uveitis) macular and peripher-al granuloma
Living Place	Coastal area, Caspian sea	Mashhad suburb
Final visual acuity	20/200	20/1000
NTU antibody titers	55.5	71

The second patient was a 5-year-old boy and he presented with strabismus in the left eye. Complete ocular examination revealed dense vitritis. He underwent vitrectomy. During the surgery, macular and peripheral granulomas were detected. All of the laboratory tests for uveitis were normal except for the elevated level of anti-*Toxocara* antibody. Serum samples were positive for anti-*Toxocara* antibody on ELISA and WB analysis. Positive WB strips indicated the presence of specific anti-*Toxocara* IgG showing multiple bands between 24 and 35 kDa (Fig. 2).

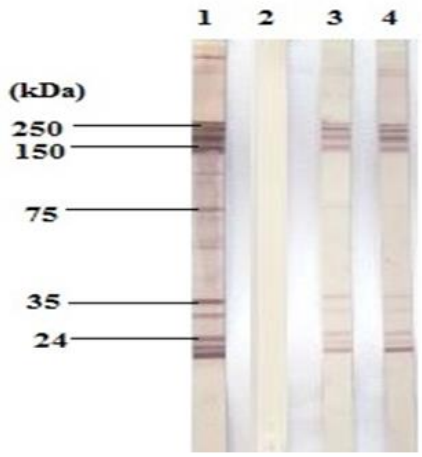

Fig. 2: WB results for sera sample of the study population. Lane 1: reference ladder; lanes 2: *Toxocara*-negative control; lane 3, 4: sera testing positive by ELISA

Discussion

This is the first study that confirms that OT can be observed in patients with uveitis in Mashhad, Iran. There have been few reports of OT from Iran (13, 14). Most of the OT cases were reported from Caspian Sea coastal areas (14). In the present study, one of patients came from this area. It means humid weather is suitable for developing eggs and larval growth as seen in similar parasite fauna.

In the present study, OT diagnosis was based on typical clinical findings and laboratory tests. Intraocular parasites or its tissue was not observed by ophthalmoscopy. The more accurate evaluation of toxocariasis in suspected patients will induce a greater number of detections of new OT patients in this region. In one study performed in this area, pet owners had significantly higher *Toxocara* antibody serum titer, compared to the control group (15). In another study, serum anti- *Toxocara* IgG titer of pet owners, living in Mashhad and suburbs, was significantly lower than those of similar study population from coastal cities of Caspian Sea (16). In another study performed in Mashhad, out of 195 soil samples obtained from public parks, 18 (9.2%) were contaminated with *Toxocara* spp. Eggs (17). The result of a seroprevalence study performed at coastal areas of Caspian Sea showed that 44% to 60% of population studies were positive against *T.*

canis and *T. cati*, respectively (18, 19). Seroprevalence of human toxocariasis base on population study indicated 15.8% to 25.6% in Iran (18). In other studies, OT was detected in both children and adults, with a mean age of 6.4 to 51.7 yr (20, 21).

Because of behavioral food habits and tendency to eat perfectly cooked meat in Iran, the prevalence of OT might be low. However, Coexistence of two different infections with *T. canis* and *T. cati* may occur due to similarity of common transmission routes, e.g., ingestion of unwashed raw leafy vegetables which is very popular in Iran (22). Iranian Muslims are not used to keeping pets especially dog and cats in their rooms and even homes.

Ocular toxoplasmosis (20% of the patients with uveitis) is more prevalent than ocular toxocariasis in Iran (23). This may be due to greater compatibility of this successful protozoan in its life cycle. The prevalence of toxoplasmosis has been higher than toxocariasis, especially in older adults.

Regarding clinical history of patient 2 (Table 2), who had long time history of direct contact with a dog, he had prolonged and severe uveitis, unilateral old posterior pole and peripheral granuloma (which was detected during vitrectomy) with positive anti-*Toxocara* IgG and negative anti-*Toxoplasma* IgG. Uveitis was inactive in this patient. It is impossible to observe larva by fundus examination, especially in old lesions. It can be identified only using microscopic evaluation of the biopsy section (24). *T. canis* larvae and *Toxoplasma gondii* had no cross-reaction on ELISA assay (25).

None of 20 highly suspected patients had history or clinical presentation of visceral larva migrans (VLM), but association of VLM and ocular toxocariasis at the same time was reported (26). Cross- reaction has been reported between *Toxocara* and some other helminths (*Trichinella, Ascaris*, *Enterobius,* etc.) (27). In this study, the two approved patients for toxocariasis, also checked by stool exam for the presence of intestinal parasites and also by WB which revealed no other infections except toxocariasis.

Different diagnostic methods have been described for OT; clinical fundus appearance, vitreous or serum ELISA, WB and PCR, using ocular tissues or aqueous fluid. Clinical presentations alone may be insufficient for diagnosis of OT. Definite and accurate diagnosis is presumptive and requires actual demonstration of *Toxocara* larvae inside the eye. In the case of absence of larva, serology tests are valuable (28). IgG inside intraocular fluids (aqueous or vitreous humour) can be detected at higher rate than with blood serum screening (29). However, obtaining intraocular fluid is an invasive technique and requires experience (20). In the present study, obtaining intraocular fluid was not possible for most of the patients (only case 2 who needed surgery). ELISA results combined with WB is highly specific for diagnosis of ocular toxocariasis (8).

In many studies, OT leads to significant visual impairment in paediatric patients (5, 30). Therefore, meticulous ophthalmic examinations and active diagnostic tests are required especially for paediatric patients with suspected OT.

This study had several limitations considered, including retrospective nature and absence of detailed ophthalmic imaging such as fundus photography and OCT. Despite the presence of tertiary eye hospital and referring many clinically suspected patients, epidemiological data of ocular toxocariasis is still unknown in Khorasan Razavi. Further study is needed regarding the prevalence, clinical features, and the diagnosis of OT at Northeastern Iran.

Conclusion

Ocular toxocariasis is an infrequent vision-threatening disease at Northeastern Iran, and more studies are needed to identify unknown cases and clear epidemiological data.

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Declaration of interest

The authors report no conflict of interest in this work.

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