



High Eosinophilia Strongly Associated with Human Toxocariasis

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Abstract

Background: The aim of this study was to reveal the prevalence of toxocariasis in patients with eosinophilia using Western blotting, and to show the importance of eosinophilia for toxocariasis.

Materials and Methods: Totally 122 individuals, of whom 37 was without eosinophilia, were included to the study and in their serum sample, presence of IgG against *Toxocara canis* was investigated by Western blotting. Patients with eosinophilia were divided into two groups. In the first group (n=52), eosinophilic cells constitute 3% to 10% of total white cells (<10%) whereas in the second group (n: 33) eosinophilic cells constitute greater than or equal to 10% of total white cells (≥10%). In addition, the cut-off value of the control group (n=37) for eosinophilia was ≤3% of total white cells.

Results: According to the results that obtained from the study, 62.3% (n=76/122) of all patients (including healthy people) were found seropositive. Also, 97% of (n=32/33) patient serum samples with ≥10 eosinophilia and 46.1% (n=24/52) of patient serum samples with <10% eosinophilia were found to be positive when patients were categorized depending on eosinophilia levels. Among control group serum samples with ≤3% eosinophilia (n=37), 54% of them were detected to be positive.

Conclusions: Accordingly, the seropositivity rate detected in patients with at least 10% eosinophilia was statistically significant when compared with the other groups (P<0.05). Seropositivity values detected in patients with eosinophilia are notable. Therefore, physicians should keep *Toxocara spp.* infection in mind in patients with eosinophilia.

Key words: *Toxocara spp.*, human toxocariasis, Western blotting, eosinophilia, *Toxocara infection*

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Introduction

Human toxocariasis is a parasitic zoonotic disease that is accidentally transmitted by ingestion of infective eggs containing *Toxocara canis* or *T. cati* and/or larvae through consumption of raw or undercooked meat and giblets (1).

It has been indicated that the risk of acquiring toxocariasis may vary depending on characteristics such as low socio-economic situation, geographic location, age, life style, and contact to dogs and cats by owners (2). The prevalence of toxocariasis among humans worldwide varies from 2.2% to 92.8%; in Turkey, it has been reported that it is between 2.16% and 51.35% (3) and in the neighboring country, Iran, ranges between 1.0 to 34.5 percent (4). On the other hand, cats and dogs are known to have an important role in transmission of toxocariasis to humans, and therefore, several studies associated with the prevalence of toxocariasis on these animals have been conducted. Results of the epidemiological studies have shown that presence of *Toxocara* species infection in cats and dogs in various parts of Turkey. The prevalence of toxocariasis in the world for *T. cati* varies from 8.0% to 91.0% in cats and 3.1% to 81.6% for *T. canis* in dogs (3).

Clinical manifestations in human toxocariasis can range from asymptomatic to systemic infections. Depending on the organs affected and the specificity of the symptoms, the predominant clinical syndromes are classified as visceral larva migrans (VLM), ocular larva migrans (OLM), neurological toxocariasis (NT), and covert toxocariasis (CT) (2, 5). Visceral toxocariasis is a serious systemic toxocariasis form characterized by high eosinophilia, hypergammaglobulinemia, fever, hepatosplenomegaly, and lung involvement, which predominantly affects children (6). Ocular toxocariasis is associated with intraocular infection and its signs are chorioretinitis, optic papillitis, endophthalmitis and keratitis, which can lead to permanent, partial or complete loss of vision (5).

In industrialized countries, total immunoglobulin (Ig)-E and eosinophilia detected at high levels often is associated with allergic diseases, whereas in developing countries, such as Turkey, this is associated with parasitic diseases (7).

Eosinophilia is known to be a clinical symptom that frequently occurs in those who have clinical symptoms associated with toxocariasis, as well as those who are *Toxocara spp.* positive but have no clinical symptoms associated with toxocariasis. High total IgE antibody response can also occur in these patients (8). In addition, antibody-dependent cell-mediated cytotoxicity is an essential defense mechanism in the process of killing larvae of parasites where persistent parasite-specific IgE play a major role.

Serodiagnosis of human toxocariasis mainly based on enzyme-linked immunosorbent assay (ELISA) which using excretory-secretory (ES) antigens released by *T. canis* larvae (2). Samples found to be positive or equivocal by the ELISA test are recommended to confirm by Western blot (WB) analysis. However, ELISA remains problematic in areas of endemic polyparasitism owing to antigenic cross-reactivity. Cross-reactivities have been reported for ascariasis, strongyloidosis, filariasis, anisakiasis, fasciolosis and trichinellosis (9-13). As a result of high seropositivity of IgG against *Toxocara spp.* and its reliability and higher specificity, Western blot may be performed as a first-line assay. The aim of study was to evaluate the importance and relationship between eosinophil levels and anti-*Toxocara* IgG by Western blot assay.

Materials and methods

Patients

Patients who applied to various clinics with allergic symptoms and were detected to have eosinophilia after laboratory examination were included in the study. Patients with eosinophilia were divided into two groups. In the first group (n=52), eosinophilic cells constitute 3% to 10% of total white cells (<10%) whereas in the second group (n: 33) eosinophilic cells constitute greater than or equal to 10% of total white cells ($\geq 10\%$) . In addition, the cut-off value of the control group (n=37) for eosinophilia was $\leq 3\%$ of total white cells. The study received ethics committee approval from İzmir Katip Çelebi University (No: 2014-34). In addition, informed consent was obtained from all participants included in the study.

Antigen preparation

Excretory-secretory antigen derived from second stage larvae of *T. canis* was produced according to Korkmaz (14). Briefly, adult female worms lysed with 5.25% sodium hypochlorite and 0.5 N NaOH and eggs collected. De-corticated eggs were incubated in 0.1 N H₂SO₄ for approximately 3 weeks, at room temperature. until the larvae motility were observed inside the eggs. Subsequently, larvae released by mechanical agitation and filtered with a modified Baermann apparatus, and cultured in RPMI 1640 (Sigma Chemical, St. Louis, MO) medium. Culture medium was collected every 3 to 4 days, pooled, and centrifuged to precipitate all debris.

Western Blotting

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Schagger and von Jagov (15). with 10% running gel and 4% stacking gel. ES antigens were solubilized in sample buffer (900 mM Tris-HCl [pH 8.45], 12% glycerol, 4% SDS, 0.005% phenol red) and electrophoresed by using Mini-Protean III electrophoresis cells (Bio-Rad). Separated antigens were transferred to nitrocellulose sheets (Protran BA 83, Schleicher & Schuell) through semi-dry electroblotting (EBU-4000, C.B.S. Scientific) for 10 minutes at 10 mA. Nitrocellulose sheets were blocked in 0.5% casein in phosphate-buffered saline, pH: 7.2 (PBS-CB). and washed four times with PBS and cut into 2-mm strips. Each serum sample prepared in 1/50 dilution by PBS-CB was incubated with a strip for 90 min and strips were washed four times using PBS. Following this step, the strips were incubated with alkaline phosphatase-conjugated goat anti-human immunoglobulin G (Sigma) diluted in 1/5000 concentration for 60 min and washed four times using PBS. Finally, antibody reactivity was visualized with 5-bromo-4-chloro-3-indolylphosphate and toluidinium-nitroblue tetrazolium substrate. All incubations were performed at room temperature on a rotatory shaker and the results were considered as positive when the samples reacted to two or more low-molecular-weight bands (LMW, 30-45 kDa). For accuracy, strips were fixed in the original position as they were cut from the membrane (16).

Results

In this study, the presence of anti-*T. canis* IgG was investigated using WB in 122 serum samples. According to the obtained results, 62.3% (76/122) of the sera samples were positive. Ninety-seven percent (n=32/33) of patient serum samples with $\geq 10\%$ eosinophilia were detected as positive. In addition, it was observed that, when the antibody response

detected in the positive samples was classified according to band intensity, 87.5% of the positive samples showed similar pattern of high positive control (Figure 1).

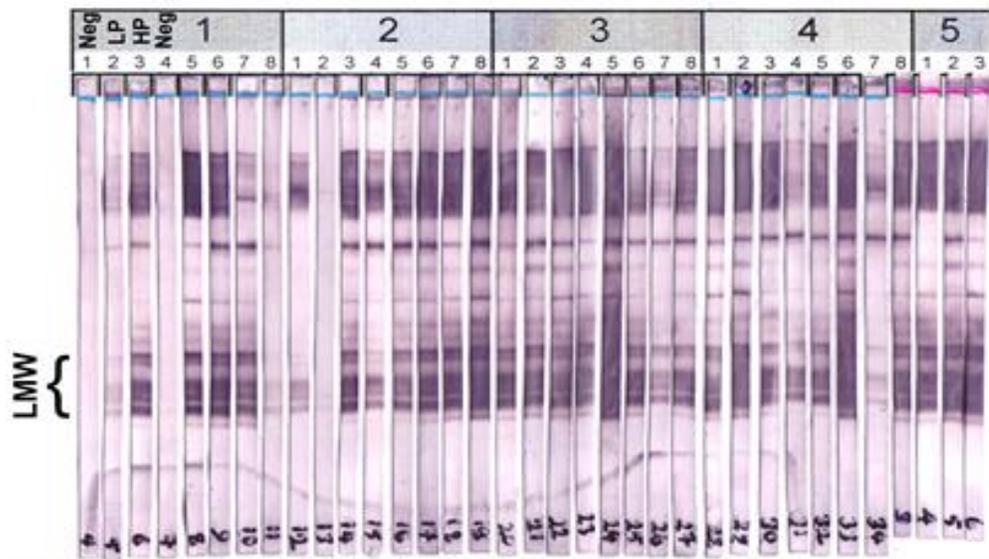


Figure 1. Demonstration of anti-*Toxocara canis* IgG response among patient serum samples with $\geq 10\%$ eosinophilia using Western blotting. Strips labelled as 1₈, 2₁, 2₂ and 4₇ evaluated as mid-level anti-*Toxocara canis* IgG response. Strips labelled as 1₄ evaluated as negative. Neg: Negative control, LP: Low positive control, HP: High positive control, LMW: Low molecular weight bands.

Of the patients with $<10\%$ eosinophilia, 46.1% ($n=24/52$) had positive for toxocariasis. When antibody levels against *T. canis* were compared among patients with $<10\%$ eosinophilia, 58.3% had high anti-*T. canis* IgG, which was compatible with the positive control serum sample, and 37.5% had mid-level anti-*T. canis* IgG. The remaining positive serum samples had low level anti-*T. canis* IgG (Figure 2A and 2B).

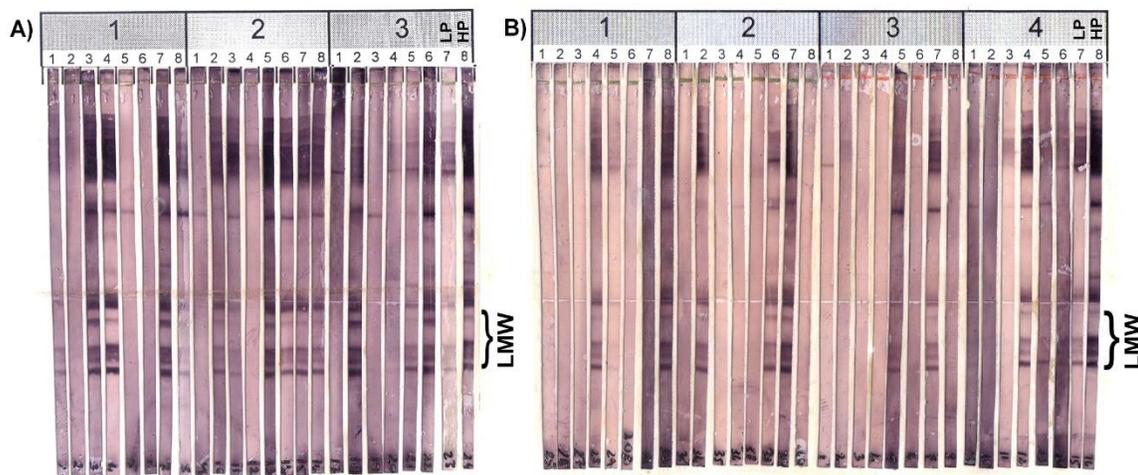


Figure 2. Demonstration of anti-*Toxocara canis* IgG response among patient serum samples with $<10\%$ eosinophilia using Western blotting. A: Strips labelled as 1₂, 1₅, 1₆, 2₁, 2₄, 3₁, 3₃, 3₄ evaluated as negative. Strips labelled as 1₁, 1₈, 2₂, 2₃, 2₆, 2₇, 2₈, 3₅ evaluated as mid-level anti-*Toxocara canis* IgG response. B: Strips labelled as 1₁₋₃, 1₆₋₇, 2₁, 2₃₋₅, 2₈, 3₁₋₄, 3₆, 3₈, 4₁₋₃ evaluated as negative. Strip labelled as 4₅ shows serum sample with mid-level anti-*Toxocara canis* IgG, (LP: Low positive control, HP: High positive control, LMW: Low molecular weight bands).

Among patient serum samples with $\leq 3\%$ eosinophilia ($n=37$), 54% of them were detected to be positive. When antibody level against *T. canis* were compared to among patients with $\leq 3\%$ eosinophilia, 50% of them had high anti-*T. canis* IgG which was compatible with positive control serum sample having high anti-*T. canis* IgG and also, 40% had mid-level anti-*T. canis* IgG. The remaining positive serum samples (10%) had low level anti-*T. canis* IgG (Figure 3).

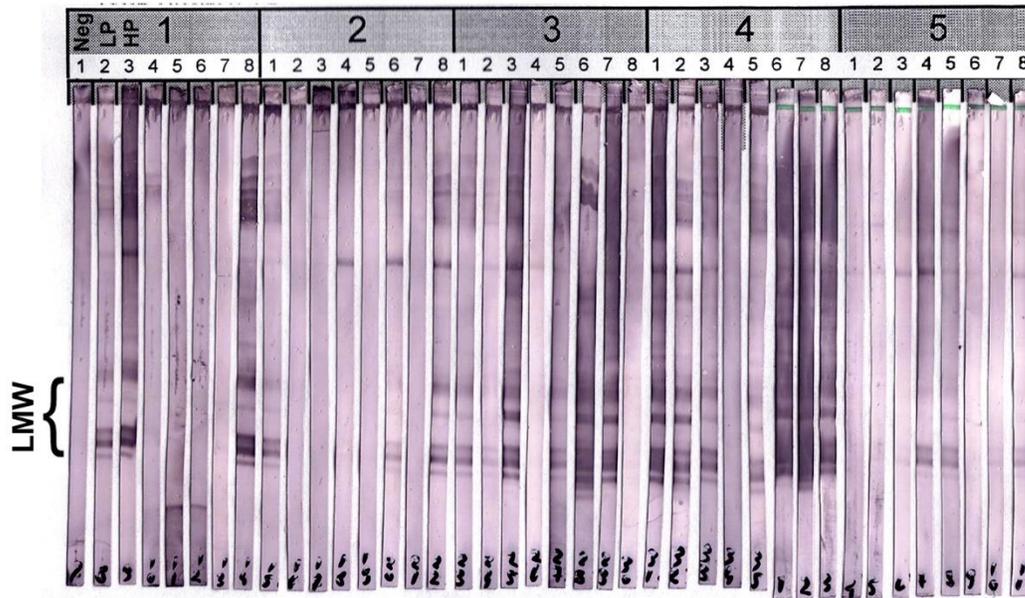


Figure 3. Demonstration of anti-*Toxocara canis* IgG response among patient serum samples with $\leq 3\%$ eosinophilia using western blotting. *Strips labelled 1₄₋₇, 2₂₋₅, 2₇, 3₄, 3₈, 4₄, 5₁₋₃, 5₆₋₇ evaluated as negative. **Strips labelled 2₁, 2₈, 3₁, 3₅, 4₅, 5₄₋₅ and 5₈ show serum samples with mid-level anti-*Toxocara canis* IgG response. ***Also, strips labelled 2₆, 3₂ represent low level anti-*Toxocara canis* IgG response.

Discussion

Toxocariasis is one of the most common parasitic zoonotic infections detected in developing countries and some tropical islands. However, because the diagnosis of infection is not well done, it is accepted that toxocariasis is among neglected parasitic diseases. In Turkey, which is a developing country, there are a limited number of studies showing the prevalence of toxocariasis in humans.

The most important or the first purpose of doing the present study was to investigate the prevalence of toxocariasis in all patients included in this study. According to the results, 62.5% of all patients were seropositive. A previous study conducted in Izmir showed that positivity rates detected in patients suspected of having toxocariasis, patients with allergy, and healthy children and adults were 39.4%, 44.9%, 28.5% and 33.3%, respectively (14). The prevalence rates of toxocariasis show great variance in Turkey. The results obtained from various studies showed that the seropositivity rate in Muğla, 8% (17), in Kayseri was 21.4% (18), in Istanbul' rural & urban areas 42.2% and 11.9% respectively (19), and in Isparta, 15.6% (20). In another study, the seropositivity rate in children living in rural areas and in urban areas of the Northwest was 16.97% and 0.71% correspondingly (21).

In our study, the seropositivity rate detected in patients with at least 10% eosinophilia was statistically significant when compared with the other groups ($P < 0.05$). This result shows that there is an association between toxocariasis and eosinophilia. Similar to our study, Rolden et al. reported that in pre-school children, there was a statistically significant relationship between toxocariasis and eosinophilia (5). In other study, the seropositivity rate was found as 68% in patients with 10% eosinophilia using ELISA (22). In a different study, the association between toxocariasis and IgE and eosinophilia was investigated and seropositivity rates were found as 69% in patients with 10% eosinophilia, 50.7% in patients with 4%-9% eosinophilia, and 40.4% in patients with <4% eosinophilia using ELISA (8). Kim et al. detected the prevalence of toxocariasis as 65.2% in patients with eosinophilia of unknown etiology (23). Also, a study showed that hypereosinophilic individuals had higher *Toxocara* infection when compared to healthy individuals (24).

Conclusion

In conclusion, although the prevalence rates of human toxocariasis show considerable differences between studies, the existing seropositivity values are notable. Physicians should keep *Toxocara spp.* infection in mind in patients with eosinophilia. The prevalence of *Toxocara spp.* infection in humans can be reduced by regularly checking for toxocariasis of stray cats and dogs and treating *Toxocara-positive* animals.

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