

# **Immunodiagnosis of Visceral Larval Migrans in Humans: Evaluation of an Enzyme-Linked Immunosorbent Assay**

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## **Abstract**

Human visceral and ocular toxocariasis can occasionally be a serious or life-threatening condition, especially for children. The diagnosis and confirmation of the disease mostly based upon serological tests. The purpose of the present study was to evaluate somatic antigen with regard to its application in a routine diagnostic laboratory test.

Using gel-filtration chromatography, fraction No.1 of *Toxocara canis* antigen (F1To) was obtained from somatic antigen of parasite, and was evaluated for the immunodiagnosis of human toxocariasis by enzyme-linked immunosorbent assay (ELISA) test. Of 30 patients with suspected clinical toxocariasis, all had anti-*T. Canis* antibodies (positive serological result) corresponding to a diagnostic sensitivity of 100%.

The specificity of the test was calculated as 77%. In this regard the positive and negative predictive values were demonstrated as 65.2% and 100% respectively. In conclusion, the application of the test using somatic antigen, because of the low specificity is not recommended.

**Keywords:** *Toxocariasis, T. canis, ELISA, Visceral larva migrans*

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## **Introduction**

Human visceral and ocular toxocariasis, which is caused by nematode larvae of the genus *Toxocara*, can occasionally be a serious or life-threatening condition (1). Among nematodes of the genus *Toxocara*, only two species, *Toxocara canis* and *T. cati* are recognized as causative agents of human Disease (2, 3). Humans become infected by ingesting either embryonated eggs from soil (geophagia, pica), dirty hands or raw vegetables, as well as larvae from undercooked giblets. The clinical spectrum of the disease comprises four syndromes, namely visceral larva migrans, ocular larva migrans, and the more recently recognized "common" (in adults) and "covert" (in children) pictures. The diagnosis and confirmation of the disease mostly based upon serological tests. In this regard ELISA test found to be very functional and authenticate.

Various investigators have used Excretory/Secretory (E/S) antigen derived from second-stage larvae of *T.canis* for the specific Immunodiagnosis of human toxocariasis by this test (4-6).

In Iran the diagnosis of the disease is mostly based upon the clinical manifestations and immunofluorescent antibody test (IFA). However, the difficulty of producing E/S antigen compared to somatic one persuaded us to challenge the validity of the ELISA test using the latter antigen. The purpose of the present study was to evaluate somatic antigen with regard to its application in a routine diagnostic laboratory test.

## **Materials and Methods**

The sera selected from patients with suspected clinical toxocariasis with antibody (IFA) against *Toxocara* antigen and who simulta-

neously had symptoms indicative of toxocariasis (eosinophilia, leukocytosis, respiratory signs, fever and hepatomegaly). It must be mentioned that, currently, only a thorough clinical examination of the cases in combination with serology is the common way to confirm diagnostically cases of toxocariasis in humans, because the direct demonstration of the parasite in tissues of humans has been proven to be unlikely (1).

The sera used for the determination of cross-reactions including hydatidosis (6 individuals), fasciolosis (6 individuals), amoebiasis (6 individuals), and toxoplasmosis (6 individuals) were obtained from patients with parasitologically, clinically or serologically proven related infections from the Tehran School of Public Health serum blood bank. They were selected from the group of sera, which was previously used for investigating the specificity of ELISA by various students. Control serum samples were obtained from 30 volunteers at Tehran University of Medical Sciences, Iran. Fraction No.1 of *T. canis* (F1To) was prepared from somatic antigen of the parasite using combination of purification methods (homogenization, sonication and ultracentrifugation) and gel filtration chromatography on cephadex G200. The immunodiagnostic assay (ELISA) was performed as previously described by Rokni et al with some modifications (7). Briefly, 100 microliters of F1To antigen (8 µg/ml) was dispensed into the wells of microtiter plates (Nuclon, Kamstrup, Roskilde, Denmark) and then incubated overnight at 37° C. Excess binding sites were blocked with 200 µl of bovine serum albumin (2% diluted in PBS/0.1% Tween 20) and incubated for 30 minutes at 37°C. After the wells were washed three times with PBS /Tween 20, 100 µl of a serum sample (diluted 1:200) was added to each plate and incubated for 60 min at 37°C. Following another washing step, 100 µl of peroxidase-conjugated goat anti-human IgG

(diluted 1:1000) was added to each well and the plates incubated for a further 60 minutes at 37°C. Following a final washing step 100 µl of O-phenyldiamine dihydrochloride (OPD) substrate (all from Sigma Chemical Co., Poole, Dorset, United Kingdom) was added to each well and the reaction stopped after 5 minutes by adding 50 µl of 12.5% H<sub>2</sub>SO<sub>4</sub>.

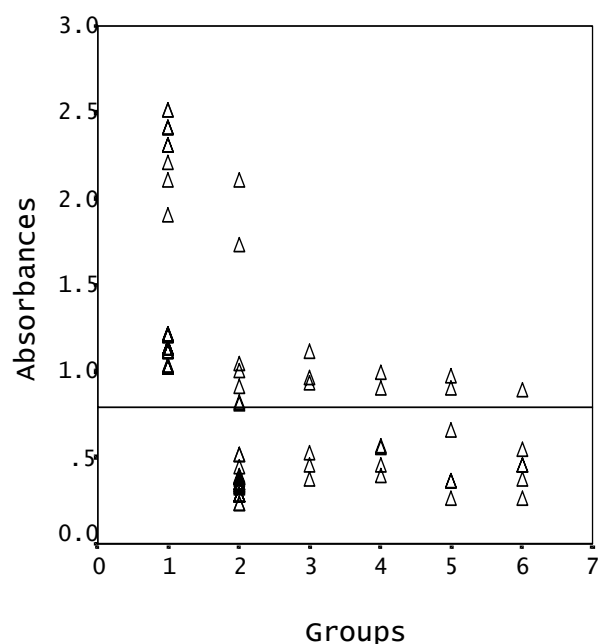
The optical density (OD) of the samples was measured at 492 nm using a Titertek (Helsinki, Finland) multiscan ELISA plate reader.

All assays were tested in triplicate and repeated twice. Statistical analysis was carried out using SPSS for windows, version 10.

## Results

Serum samples obtained from 30 individuals infected with toxocariasis were analyzed by ELISA for total antibody responses against F1To antigen. Using hierarchical agglomerative cluster analysis the cut-off points between clusters was 0.789 for F1To antigen. Therefore absorbance readings greater than the cut off value were considered to be seropositive for Toxocariasis.

Accordingly, all 30 individuals that showed clinical manifestations of toxocariasis and were confirmed using IFA test were also seropositive using F1To antigen whereas 23 out of 30 non-infected controls clustered together as seronegative. Therefore, the sensitivity of the test was detected as 100%. However, some cases of non-toxocariasis infected people had antibodies that were reactive against *T. Canis* F1To antigen (Figure 1), moreover 7 cases of control group showed seropositive in the test. For the present data set, therefore, the specificity of the test was calculated as 77%. In this regard the positive and negative predictive values were demonstrated as 65.2% and 100% respectively.



**Fig. 1:** Analysis of sera from patients with various single infections by IgG-ELISA using *T. canis* F<sub>1</sub>To as antigen. Serum samples obtained from patients with toxocariasis (30, lanes 1), control human sera (30, lanes 2), fasciolosis (6, lanes 3), hydatidosis (6, lanes 4), amoebiasis (6, lanes 5), and toxoplasmosis (6, lanes 6).

## Discussion

Toxocariasis still is an important infection in the world and in Iran, particularly as the greatest burden of disease falls on children (8). In our country this disease is diagnosed mostly by clinical manifestation and IFA test. There is need for a more valuable test to be evaluated and established. As the preparation of E/S is difficult and time consuming, we determined to challenge the use of somatic antigen in this regard. The present report demonstrates the use of *T. canis* F<sub>1</sub>To antigen for the diagnosis of toxocariasis. As is shown in Figure 1, antibodies in the serum of some cases with different non-toxocariasis diseases were reactive with F<sub>1</sub>To antigen. Polyparasitosis, where an individual may harbor two or three parasitic infections at the same time, is common in the region (9) and raises the possibility that these patients were also exposed to toxocariasis. This phenomenon can complicate

the interpretation of less specific serological tests (6, 10). Moreover, somatic antigen, encompasses multiple epitopes, could not be numerated as a suitable antigen all the times. However, some investigators demonstrated that when E/S was used in the ELISA, the validity of the assay increased markedly (4-6). Glickman et al. (11) has reported sensitivity, specificity and positive and negative predictive values of 78.3%, 92%, 85% and 85% respectively, for ELISA test using larva E/S antigen. The ELISA test quantifies the serological response, and this may lead to difficulty in interpretation (12). It has been suggested that antibodies to E/S antigens are more likely to be related to active migration and infection than are antibodies evoked by somatic antigens- for example in the indirect fluorescent antibody test- and therefore indicate a truer causal relation (12). Non the less it is stated that antibody to E/S antigens persisted for a long time (6) and other researchers have confirmed such a comment more or less (13). Clearly further work is required on the establishment of a valuable diagnostic test and as stated in the text, because of the low specificity of somatic antigen we recommend that investigations must be focused on using of E/S antigen in ELISA test.

## Acknowledgements

We would like to show appreciation to Miss M. Roohnavaz, Mr Ali Rahimi and Mr Shams from the department of Parasitology, School of Public Health, Tehran University of Medical Sciences, Iran, for their sincere cooperation.

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