Original Article

Clinical, Laboratory Characteristic, and Treatment of Human Toxocariasis in Northern Vietnam

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Abstract
Background: We determined the occurrence frequency of Clinical signs, subclinical characteristics on positive anti-IgG Toxocara ELISA cases, and clinical response results after 1 month of albendazole-specific treatment.

Methods: A number of 103 clinically treated patients were studied between 2018 and 2019 in two hospitals. Relevant data are collected from examination and medical records such as age, sex, job, resident, clinical and sub-clinical signs. Sera samples were subjected to anti-IgG Toxocara antigen using ELISA.

Results: The median age of the patient was 43.6 ± 14.4 years. The most common symptom groups were the skin and mucosa (88.3%), followed by neurological symptoms (44%). There are 76.7% of patients with risk factors for contact with pet dogs and cats. The majority of eosinophils in the normal range (< 8%) accounted for 85.4%, the major results of the OD value of ELISA were in the group (≥ 0.3 - < 1.5) accounted for 75.7%. The skin and mucosa lesions in toxocariasis patients related to IgE values were statistically significant (P <0.05). The effectiveness of anthelmintic treatment is low with nearly half of patients having no clinical symptoms after 1 month of post-treatment. It is statistically significant between exposure to dogs/cats and treatment outcomes on clinical symptoms (P < 0.05).

Conclusion: Toxocara infections in Vietnamese people often have skin allergies and liver damage, rarely with severe neurological symptoms. The efficacy of albendazole treatment after one month is not clear. In the near future, research with a confirmatory test other than Anti-IgG Toxocara ELISA is needed to screen for in-hospital diagnosis.

Keywords: Toxocariasis; Visceral larva migrants; Serology

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Received 21 Apr 2021
Accepted 11 Jun 2021
Introduction

Human toxocariasis is a zoonosis caused by the ingestion of embryonated *Toxocara* eggs from contaminated soil and, or infective larvae in raw/undercooked meats (1). In 1952, Beaver et al. proved the presence of *Toxocara* larvae in humans and called it "visceral larva migrants" disease (2). There are two species, *T. canis*, and *T. cati*, recognized as causative agents of human toxocariasis (3).

*Toxocara* spp. infection in humans is usually asymptomatic but it can also cause damage to various organs with diverse clinical manifestations as the larvae migrate in human organs or reside locally (4). Moreover, the clinical symptoms of dog roundworm and cat roundworm larvae infection are relatively similar and most modern tests have not been able to distinguish clearly these two species (5).

Vietnam is a developing and tropical country with an agricultural economy. In addition, a large part of the Vietnamese often lives close to pets (dogs/cats) and with specific eating behavior and consumption of raw foods such as sour meatball, blood pudding, etc., which are favorable conditions for zoonosis, including toxocariasis (6). In Vietnam, there are few studies on *Toxocara* spp. infection status as well as clinical, subclinical, and treatment characteristics of toxocariasis with a focus on epidemiological features (7-11).

We conducted the study with the objectives to determine the frequency of occurrence of clinical signs, subclinical characteristics, and their association with positive anti-IgG *Toxocara* ELISA response in patients.

Materials and Methods

Study design and Population

A cross-sectional study was performed on all clinical samples infected with *Toxocara* and received outpatient treatment with albendazole at the Outpatient Department, Military Hospital 103 (MH103) and inpatient treatment at the Clinical Department of the National Institute of Malariology, Parasitology, and Entomology (NIMPE) from October 2018 to October 2019.

A total of 103 patients aged 16 years and older agreed to participate in the study meeting the diagnostic criteria of *Toxocara* in humans, including at least two clinical symptoms and positive for anti-IgG *Toxocara* ELISA considering the existing protocols (12).

Demographic data (age, sex, residence address, occupation and clinical, subclinical, and other clinical follow-up results) are taken from medical records as well, including Optical Density (OD) results of ELISA test for *T. canis* antibodies and various clinical symptoms.

This study was approved by the Ethical Committees of Vietnam Military Medical University (No.150/2018/IRB-VMMU). All patients provided written informed consent, and that this study was conducted in accordance with the Declaration of Helsinki.

Extraction of clinical signs

Patients in the study were asked to detect detailed medical history. They were examined physically completely and systematically. They were designated to take necessary tests and were specifically recorded according to a separate research sample of medical records. We collected general characteristics of the subjects: age, gender, occupation, living area, phone number, date of examination, admission code. Medical history has selected the reasons for admission, duration of illness before, history, and epidemiological factors. Physical examination (15) was performed to identify clinical signs according to each group of systemic syndromes. The clinical data collected as skin syndrome (such as
maculopapular rash, papules, itching, chronic rashes, chronic urticaria); Neurological symptoms (such as headache, epilepsy, numbness), systemic symptoms (such as aches and pains fever, digestive system, swollen glands, edema or anemia); Respiratory symptoms (such as dry cough, wheezing, shortness of breath, rale) and Digestive symptoms (such as abdominal pain, nausea/vomiting, diarrhea, hepatomegaly or splenomegaly).

Detection of anti-Toxocara IgG
From 2018 to 2019 October, the information recorded of the patients' records (such as OD) has been used and a new ELISA has been performed all the Toxocara VLM suspected cases. These cases were re-tested by an anti-IgG Toxocara ELISA diagnostic kit (Cortez Diagnostic Co. ltd. USA, lot number DA1872) in our laboratory. According to the manufacturer, the kit has a sensitivity of 93.3% and a specificity of 87.5%. The OD at double ways 450/650 nm was measured with an automated ELISA Zero reader. An OD value less than or equal to 0.2 was considered as negative and that greater than or equal to 0.3 was considered positive. While the values within this range (0.2 < OD < 0.3) were considered as a light infection or past infection, the OD value > 0.5 was considered as high positive indicating recent infection (13,15).

Biochemical and hematological indicators
A differential white blood cell count was performed to determine the percentage of eosinophils on the 22-parameter hematology. Eosinophils usually account for less than 8% of the circulating white blood cells (100 to 500 eosinophils per microliter of blood [0.1 to 0.5 × 10⁹ per liter ⇄ 0-8%]) (13). We divided the group with the eosinophil count increased from 8% or more us and the group lower than 8%.

Furthermore, samples were analyzed for levels of serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) (AU680, Beckman Coulter, Brea, CA, USA). Elevated AST and ALT levels were defined as AST and ALT levels above 35 IU/L and 56 IU/L, respectively (17). With the biological kit provided by the supplier at the time of the study, these increased indicators were set to AST > 40 IU/L, ALT > 40 IU/L, and GGT > 50 UI/L (12).

The allergen-specific immunoglobulin E (IgE) test is a test that measures different levels of IgE antibodies in the blood. According to the IgE test kit instructions, a concentration of > 35 UI/mL is a positive case, but when IgE ≥100 is an extremely strong positive (18). Research subjects are adults, so we evaluate this index in 2 groups of IgE 100 and <100 UI/mL (13).

Abdominal ultrasound
Many pore-shaped nodules where liver parenchyma was damaged secondary.

Treatment results of toxocariasis patients
Patients with Toxocara who fully met the study criteria were treated with albendazole 800 mg/day for 14 days (12,19). As all the patients in the study were adults, their weight ranged from 50-70 kg, the albendazole dose for treatment in the study was 800 mg/day. Evaluating the treatment results after 1 month based on the remaining or finish of clinical manifestation (such as systemic, skin, respiratory, digestive, neurological symptoms), and change of several subclinical indexes, in particular eosinophils.

Statistical analyses
Data were imported in Excel (Microsoft Office Excel, 2010). The Intercooled Stata™ Statistical Package (StataCorp LLC, College Station, TX, USA) was used for statistical analysis. Two-variable analysis of the data set used Pearson's χ² and Fisher's tests for categorical variables and Student's t-tests for continuous variables. In addition, one-way
ANOVA was applied to serological categories and mean age to confirm the above results with an approximate 95% confidence limit (CI95%, P-value < 0.05).

**Results**

**General characteristics of the study population**

A total of 103 participants were involved in the study. The distribution of human Toxocariasis rate by age group, gender, occupation, place of residence, and contact with pets is presented in Table 1.

| Characteristics                  | No. (n=103) | %  |
|----------------------------------|-------------|----|
| Age groups (yr)                  |             |    |
| 16 - < 30                        | 21          | 20.4 |
| 30 - < 45                        | 32          | 31.1 |
| 45 - < 60                        | 33          | 32  |
| ≥ 60                             | 17          | 16.5 |
| Mean ± SD (Min-Max)              | 43.6 ± 14.4 (19 - 74) |
| Sex                              |             |    |
| Male                             | 50          | 48.5 |
| Female                           | 53          | 51.5 |
| Local living                     |             |    |
| Urban area                       | 52          | 50.5 |
| Rural area                       | 42          | 40.8 |
| Mountainous area                 | 9           | 8.7 |
| Occupation                       |             |    |
| Workers                          | 10          | 9.7 |
| Officers                         | 36          | 35.0 |
| Farmers                          | 15          | 14.6 |
| Business                         | 9           | 8.7 |
| Students                         | 5           | 4.9 |
| Others                           | 28          | 27.1 |
| Contacting with pets             |             |    |
| Yes                              | 79          | 76.7 |
| No                               | 24          | 23.3 |
| Reasons for medical examination  |             |    |
| Rash/Itchy urticaria             | 91          | 88.3 |
| Fever/Aches & Pains              | 19          | 18.4 |
| Dry cough/Wheezing               | 12          | 11.7 |
| Headache/Numbness in limbs       | 45          | 44.0 |

**Clinical and subclinical characteristics of toxocariasis patients**

The results of monitoring clinical and subclinical signs in the pre-treatment of 103 patients and post-treatment of 54 patients are shown in Tables 2-4.
### Table 2: Clinical characteristics of *Toxocara* patients' pre-and post-treatment

| Clinical signs                   | Pre-Treatment | Post-Treatment | P-value |
|---------------------------------|--------------|----------------|---------|
|                                 | No. (%       | No. (%)        |         |
| Skin and mucosa signs:          | 91 (88.3)    | 19 (35.2)      | < 0.05  |
| + Maculopapular rash            | 28 (27.2)    | 0 (0.0)        | < 0.05  |
| + Papules                       | 67 (65)      | 7 (13.0)       | < 0.05  |
| + Itching                       | 89 (86.4)    | 19 (35.2)      | < 0.05  |
| + Chronic rashes               | 7 (6.8)      | 3 (5.6)        | > 0.05  |
| + Chronic urticaria             | 3 (2.9)      | 2 (3.7)        | > 0.05  |
| Neurological symptoms:          | 45 (44)      | 11 (20.4)      |         |
| + Numbness                      | 38 (36.9)    | 8 (14.8)       |         |
| + Headache                      | 35 (34)      | 11 (20.4)      |         |
| + Epilepsy                      | 0 (0)        | 0 (0.0)        |         |
| Systemic symptoms:              | 19 (18.4)    | 3 (5.6)        | < 0.05  |
| + Aches and pains               | 19 (18.4)    | 3 (5.6)        | < 0.05  |
| + Fever                         | 9 (8.7)      | 2 (3.7)        | > 0.05  |
| + Swollen glands                | 1 (1)        | 0 (0.0)        |         |
| + Edema or Anemia               | 0 (0)        | 0 (0.0)        |         |
| Respiratory symptoms:           | 12 (11.7)    | 6 (11.1)       | > 0.05  |
| + Dry cough                     | 12 (11.7)    | 6 (11.1)       | > 0.05  |
| + Wheezing                      | 5 (4.9)      | 0 (0.0)        | > 0.05  |
| + Shortness of breath           | 1 (1)        | 0 (0.0)        |         |
| Digestive symptoms:             | 11 (10.7)    | 5 (9.3)        |         |
| + Abdominal pain                | 11 (10.7)    | 5 (9.3)        | > 0.05  |
| + Nausea/Vomiting               | 9 (8.7)      | 3 (5.6)        | > 0.05  |
| + Diarrhea                      | 8 (7.8)      | 2 (3.7)        | > 0.05  |
| + Hepatomegaly or               | 0 (0)        | 0 (0.0)        |         |
| + Splenomegaly                  | 0 (0)        | 0 (0.0)        |         |
| Total                           | 103 (100)    | 54 (100)       |         |

### Table 3: Biochemical and hematological indicators of *Toxocara* patients

| Sub-Clinical signs               | No. (%       |         |         |
|---------------------------------|--------------|---------|---------|
| White blood cell                | 103 (100)    |         |         |
| < 4 G/l                         | 2 (1.9)      |         |         |
| 4 - 10 G/L                      | 90 (87.4)    |         |         |
| >10 G/L                         | 11 (10.7)    |         |         |
| Mean ± SD                       | 7.3 ± 2.4    |         |         |
| Eosinophil rate                 | 103 (100)    |         |         |
| < 8 %                           | 88 (85.4)    |         |         |
| ≥ 8 %                           | 15 (14.6)    |         |         |
| Mean ± SD                       | 4.1 ± 3.3    |         |         |
| Liver enzymes                   |              |         |         |
| AST > 40 U/l                    | 11 (10.7)    |         |         |
| ALT > 40 U/l                    | 19 (18.4)    |         |         |
| GGT > 50 U/l                    | 22 (21.4)    |         |         |
| IgE value                       | 17           |         |         |
| ≤ 100 UI/mL                     | 4 (23.5)     |         |         |
| > 100 UI/mL                     | 13 (76.5)    |         |         |
| Mean ± SD                       | 532.1 ± 493.5|         |         |
| min-max                          | 19.6 - 1,674 |         |         |
| ELISA *Toxocara canis* IgG      | 103          |         |         |
| 0.3 ≤ OD < 1.5                  | 78 (75.7)    |         |         |
| 1.5 ≤ OD                        | 25 (24.3)    |         |         |
| Mean ± SD                       | 1.2 ± 0.8    |         |         |
| abdominal ultrasound            | 27           |         |         |
| Liver damage                    | 9 (33.3)     |         |         |
| Normal                           | 18           |         |         |
Table 4: Relationship between eosinophil index, IgE with skin symptoms and risk factor of Ab-ELISA *Toxocara* positive cases

| ELISA positive and Contacting with pet | No/n | %   | OR   | P-value |
|---------------------------------------|------|-----|------|---------|
| Eosinophil ≤ 8 %                      | 12/79| 15.2|      |         |
| Eosinophil ≥ 8 %                      | 3/24 | 12.5| 0.82 | >0.05   |
| Eosinophil normal                     | 79/88| 89.8|      |         |
| Eosinophil increase                   | 12/15| 80.0| 0.89 | >0.05   |

**Treatment outcomes of albendazole therapy in toxocariasis patients**

In our study, 103 cases were diagnosed with human toxocariasis and were treated with albendazole 800 mg/day for 14 days. However, after 1 month of treatment, we only recorded 54 follow-up cases. All 54 cases re-examined complete blood count and 31 cases were re-tested ELISA. The percentage of patients finishing clinical symptoms after 1 month of treatment was 48.1%, while that of remaining clinical symptoms accounted for 51.9%. As the OD value of the ELISA test of patients after 1 month of treatment does not change much and does not make significance, we focused on evaluating the results of treatment based on changes of clinical symptoms and eosinophils values in Table 2 and 5.

Table 5: Results of follow-up tests post-treatment in *Toxocariasis* patients

| Index                        | Treatment outcomes | p      |
|------------------------------|--------------------|--------|
|                              | Remaining CS (n=28) | Finishing CS (n=26) | Total CS (n=54) |
|                              |                    |                    |                  |
| Contacting with dogs or cats | Yes                | No. (%)            | 50.0             | <0.05 |
|                              | No                 | No. (%)            | 50.0             | <0.05 |
| Eosinophil index (n=54)       | Increase (≥ 8%)    | No. (%)            | 50.0             | <0.05 |
|                              | Normal (<8%)       | No. (%)            | 50.0             | <0.05 |
| OD Ab-ELISA (n=54)            | ≥0.3-<1.5          | No. (%)            | 50.0             | <0.05 |
|                              | ≥1.5               | No. (%)            | 50.0             | <0.05 |
| IgE value (n=12)              | Increase (>100 UI/ml) | No. (%)          | 50.0             | <0.05 |
|                              | Normal (≤100 UI/ml) | No. (%)          | 50.0             | <0.05 |

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Discussion

Toxocariasis represents one of the most common parasitic zoonotic infections worldwide particularly in developing countries and some tropical islands (17,18). In Vietnam, common adult Toxocara diseases have been reported (6-11).

In this study, the average age of Toxocara-infected patients was higher than in previous studies (43.6 ± 14.4 years old), contributing to reduced working capacity. The incidence of males was equivalent to that of women (7, 20). The rate of patients living in urban areas accounted for 50.5%, followed by rural areas with 40.8% and only 8.7% of patients lived in mountainous areas. People get infected toxocariasis not only in rural areas with difficult economic conditions but also living in urban areas, the incidence was similar, relatively high. It is possible that due to the study at two hospitals in Hanoi City, the distribution of patients is up to half who are urban (7,8). The rate of toxocariasis infection varies between occupations, in which the majority of the group were civil servants (22). Taking a closer look at the reasons why this group infected with Toxocara was higher than other occupational groups, we found that most of those cases were pets. This was a risk factor for increasing the prevalence of dog and cat roundworm larvae in humans.

The cause of discomfort for patients going to the doctor is usually an allergic reaction, itching, and a red rash on the skin. These are the most common symptom (88.3%) and also easy to confuse the diagnosis with an allergy caused by another allergen (Table 1,2). Therefore, elevated IgE tests, eosinophils index were less associated with toxocariasis (Table 3-5).

Visceral larva migrants were first described in 1952, in children with an enlarged liver and hypereosinophilia (2). The typical VLM patient is a child between the ages of 2-7 years with a history of geophagia and exposure to puppies in the home (23). The same evidence suggests that Vietnamese are susceptible to Toxocara disease in adults with allergic skin manifestations and liver damage (6-11). Peripheral blood eosinophilia, although not specific for Toxocara infection, is associated with VLM (3).

Up to now, the subclinical diagnosis is still mainly based on ELISA immunoassay using antigen secreted from toxocariasis larvae. Some authors have noted that respiratory symptoms such as cough are common in children with Toxocara ELISA-positive results. In addition, there are indications that the cases in Vietnamese have milder symptoms than the reports.

The benzimidazole derivatives, mebendazole (MBZ), and albendazole (ABZ) are used in the treatment of roundworm diseases in animals and humans. However, both MBZ and ABZ are poorly absorbed from the gastrointestinal tract and should be taken with a fatty meal (3). In Vietnam according to the guidelines of the Ministry of Health and NIMPE, the national institute specializing in parasitology, Toxocariasis treats adults according to the albendazole 800 mg/day regimen for 14 days (12, 19). Patients will be prescribed outpatients and 1 month after treatment with specific drugs, they will be re-examined, re-evaluated for further clinical follow-up directions. The results (Table 2&5) for a month post-treatment only about half of the patients examined (54/103) and only nearly half (48.1%) had no certificates.

For post-treatment follow-up by eosinophil counts, total IgE and ELISA are useful (3). In this controlled trial of toxocariasis with ALB there was no significant reduction in mean blood eosinophil counts and anti-Toxocara antibodies within 1 month of treatment, while total serum IgE levels, there was no significant change in serum (Table 3,5). Although, in the clinically symptomatic group with high Toxocara antigen levels, the total IgE concentration was significantly higher (Table 5). Thus, the
detection of specific anti-\textit{Toxocara} IgG by ELISA is unlikely to be useful for monitoring treatment. When comparing pre- and post-treatment ELISA antibody titers, the kinetics of anti-\textit{Toxocara}-specific IgG were not affected by deworming treatment (3). The best alternative for serological diagnosis of screen toxocariasis, visceral migratory larva, is based on the use of Ab-ELISA initially followed by TES – ELISA. After that, any positive results will then be checked by the western blotting method (WB). In Vietnamese hospitals, WB has not been applied in clinical practice. This is also a limitation for the diagnosis of infectious parasitic diseases at present and is a new direction for applied research.

The factor of contact with pets had a relationship with the treatment outcomes on clinical symptoms and the difference was statistically significant ($P<0.05$). Our treatment results were higher than that of toxocariasis patients in a study conducted in the Central Highlands region, with one of the fourth patients having no symptoms after 1 month of treatment with an albendazole 3 weeks regimen (9-11).

This study was limited with a small sample and did not necessarily represent the study population, so it is difficult to generalize research results. ELISA results collect data from medical records, so there may be technical heterogeneity. The short follow-up time of post-treatment parameters is not enough to evaluate treatment efficacy. The sample may not be representative of ages, races, and places of residence, so the analysis of risk factors is much limited.

**Conclusion**

The disease is at working age, there is no difference in gender and place of residence; Skin symptoms are the main and few neurological symptoms, liver damage. There are signs of illness related to contact with the pet. The efficacy of the drug is not high and unclear after a one-month albendazole cure. The ELISA Anti-IgG \textit{Toxocara} test is used as a screening tool to recommend hospital diagnostics, but not for monitoring response to treatment, case-by-case.

It is necessary to have a technique with better sensitivity and specificity than the diagnostic serum for detecting antigen \textit{Toxocara} at hospital facilities to examine and confirm the diagnosis of this parasitic disease group. Effectiveness after 1 month of treatment is low (48.1%), it is necessary to have research direction to change the treatment regimen.

**Acknowledgments**

The authors also sincerely thank Professor Hoang Vu Hung, the Head of the Department of Infectious Diseases, Military Hospital 103, for his suggestions on project construction ideas and implement the project. A deep thank you to the colleagues at the Outpatient Department, Military Hospital 103, and at the Clinical Department of the National Institute of Malariaology, Parasitology, and Entomology Hanoi, Vietnam, and for their cooperation in the implementation of the project. Many thanks to the patients and their families who enthusiastically participated during the implementation of this project. The author(s) received no financial support for the research, authorship, and/or publication of this article.

**Conflict of interest**

The authors declare no potential conflicts of interest to the research, authorship, and/or publication of this article.

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