

Clinico-Seroepidemiological Evaluation of Toxocariasis in Asthmatic Egyptian Children

Samir A. Shahat¹, Khaled Abd El-Aziz Mohammad², Mohammed Youssef Saad², Tarek M. Emran³,
Soma Abdallah Mohamed⁴, Mahmoud Elsayed Ali Elshahat²

¹Department of Medical Parasitology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

²Department of Medical Parasitology, ³Department of Clinical Pathology, Faculty of Medicine, Al-Azhar University, Damietta, Egypt.

⁴Department of Pediatrics, Faculty of Medicine (girls), Al-Azhar University, Cairo, Egypt

Corresponding author: Mahmoud Elsayed Ali Elshahat, e-mail: ma536552@gmail.com

ABSTRACT

Background: Toxocariasis was recorded as one of the most commonly zoonotic helminthic infections in the world and still a poorly diagnosed disease hence largely unknown either to health professionals and/or the general of Egyptian population. The excretory-secretory antigens of *T. canis* larvae (TES) are widely used for both the diagnosis and seroepidemiological studies. **Aim of the work:** The aim of the present study was to assess the relationship between toxocariasis and bronchial asthma in asthmatic children through means of a case-control study in Cairo, Egypt and to determine its relation to epidemiological risk factors, laboratory tests and clinical signs.

Patients and Methods: The study included two groups (Group1): asthmatic group included 72 children with confirmed asthmatic bronchitis and (Group 2): non-asthmatic group included 72 children selected randomly as a control group. The sociodemographic data was assessed as risk factors for toxocariasis based on a questionnaire collected from the children's parents or guardians. The selected cases were subjected to stool analysis to exclude other parasitic infection, CBC for eosinophilia and immunoblot assay for detection of *Toxocara canis* IgG.

Results: Out of 72 asthmatic patients toxocariasis IgG was positive in 16 cases (22.2%), negative in 56 cases (78.6%) compared to 5 positive cases (6.9%), and 67 negative cases (93.1%) in control group. There was a significant relation between positive anti*Toxocara* IgG and asthmatic bronchitis. This study confirmed a significant correlation between the seroprevalence of *Toxocara* and possible socioepidemiological factors as contact with pets, geophagia and residence.

Conclusion: Rising knowledge of toxocariasis will enable pediatricians to consider it as a cause of asthmatic bronchitis in their patients, initiate appropriate treatment, and educate patients and parents on how to avoid becoming infected.

Keywords: children, bronchial asthma, toxocariasis, seroprevalence, immunoblot IgG.

INTRODUCTION

Toxocariasis is caused by two species of the ascarid nematodes *Toxocara canis* and to a lesser extent *T. cati* which is considered as one of the most commonly zoonotic disease. Also, *Toxocara leonina* which cause mixed infections in cats and dogs can be able to infect humans; therefore, it has zoonotic and public health importance ⁽¹⁾.

Accidental intake of embryonated eggs of *Toxocara canis* or *Toxocara cati*, (the intestinal parasites of dogs and cats, respectively) is the source of infection to humans where only few larvae are needed to cause the disease ⁽²⁾. In humans the primary route of transmission of *Toxocara* is contact with contaminated soil ⁽³⁾, in addition to, direct contact with dog carrying eggs in their fur ⁽⁴⁾. Also, another different route of transmission involves consumption of raw vegetables grown in contaminated gardens, geophagia as well as persons with poor hygiene ⁽⁵⁾.

No clinical manifestation occurred in most cases when infected larvae migrate through the human tissues ⁽⁶⁾. However, visceral larva migrans is induced (VLM) in parasitized individuals with the presence of anti-*Toxocara* antibodies, which affects mainly young

children and characterized by leukocytosis, hypergammaglobulinemia, fever, hepatomegaly, cough, wheeze, abdominal pain, and elevated serum IgE level ⁽⁷⁾. Eosinophilia is one of the most outstanding characteristic of the VLM in naturally infected humans and experimental models of infection ⁽³⁾.

Humans in the life cycle of *Toxocara* are considered as paratenic hosts in which the larvae cannot develop into adult worms and therefore, the parasitological examination of feces does not contribute to the laboratory diagnosis ⁽⁸⁾. The diagnosis of toxocariasis is generally based on clinical signs and symptoms, which are non-specific, epidemiological data (contact with dogs or cats, geophagia, onychophagy, consumption of undercooked or raw meats) and laboratory findings ⁽⁹⁾.

The immunoblot or Western blotting assay is a test that combines the high sensitivity of the immunoenzymatic tests with the high resolution of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE). This method has been successfully adapted for the confirmatory serodiagnosis of various parasitic diseases, including toxocariasis, schistosomiasis, hydatidosis, cysticercosis, taeniasis, fasciolosis and strongyloidiasis ⁽¹⁰⁾.

Nowadays, in patients with suspected toxocariasis, immunoblot assay is useful to confirm any positive serum by the ELISA test (where pre absorption is not carried out) ⁽¹¹⁾.

PATIENTS and METHODS

1. Study population: This study was carried out in the Medical Parasitology Department, Damietta faculty of Medicine, Al-Azhar University and the Pediatric Department (inpatient and outpatient) of Al-Zahraa Hospital, Faculty of Medicine for girls, Al-Azhar University on asthmatic children from September 2017 to April 2018. The study included two groups, (**Group1**): asthmatic group included 72 children (42 males and 30 females) with confirmed asthmatic bronchitis and (**Group 2**): non-asthmatic group included 72 children (39 males and 33 females) selected randomly as a control group.

The age of the children in both groups ranged from 3 to 15 years old with different socioeconomic and educational levels. An informed written consent was obtained from their parents or guardians. This study was approved by the local ethical committee of Faculty of Medicine Al-azhar University. Asthmatic patients were diagnosed according to the GINA Report 2018, Global Strategy for Asthma Management and Prevention ⁽¹²⁾.

The sociodemographic data was assessed as age, sex, contact with pet-animals and residence (urban or rural). The selected cases were subjected to stool analysis for parasitic infection, CBC for eosinophilia and immunoblot assay for *Toxocara canis* IgG.

2. Stool analysis: Fecal samples were collected from each subject in a clean, dry plastic container. The feces were macroscopically examined for color, consistency, mucus and presence of worms or parts of them. Then, stained slides, that prepared through direct smear and formalin-ether concentration techniques were examined microscopically by low and high powers to detect other helminthic ova, larvae, trophozoites and cysts where positive cases were excluded to avoid cross-reactions.

3. Complete Blood Count (CBC) for eosinophilia: Blood samples were collected individually; three ml of venous blood were collected in 2 tubes, one with EDTA as anticoagulant for detection of eosinophilia which is the increase of the blood eosinophils by $\geq 450/\mu\text{l}$ ⁽¹³⁾. The second tube was centrifuged to separate sera which were stored at -20°C until needed for anti-*Toxocara* IgG using immunoblot assay. Total and differential leucocytic count were assessed using (Sysmex XP-300™ Automated Hematology Analyzer).

4. Immunoblot assay for *Toxocara canis* IgG: *Toxocara* Western Blot (WB) IgG (LDBIO Diagnostic, 19A rue Louis loucher, Lyon, France -

www.ldbiodiagnostics.com) is a qualitative test for detection of IgG antibodies against the excretory/secretory antigen of the *Toxocara* larvae. The excretory/secretory (E/S) antigens of *Toxocara canis*, once separated by electrophoresis, are bound by electro blotting to the surface of a nitrocellulose membrane (called the transfer) cut into 24 strips numbered from 1 to 24. Each serum sample to be tested is separately incubated with a strip. The anti-*Toxocara* antibodies potentially present in the sample selectively bind themselves onto the E/S antigens of *T. canis*. The alkaline phosphatase-anti human IgG conjugate then binds itself to the bound anti-*Toxocara* antibodies. Finally, the immunocomplexes react with the substrate. The antigens recognized by the anti-*Toxocara* antibodies of type IgG present in the samples are revealed as purple transversal band.

A minimum of 10 μl of serum is needed. We prepare a distribution plan for samples and C+ positive control (R10). We distribute 1.2 ml of sample buffer (R2) in each channel according to the established plan. Let the strips rehydrate themselves for approximately 1 minute, with the number visible at the top, by gently shaking the tray to totally immerse them in the buffer. We distribute the samples and positive control (s): according to the distribution plan, at a rate of 10 μl per channel. Gently shake the tray after each dispense. Place the tray on a rocking platform. Incubate for 90 min \pm 5 min at 18-25 °C.

Wash step: the contents of the channels were emptied by turning the incubated tray over. Two ml of diluted wash buffer were dispensed in each channel then incubated on the rocking platform for 3 min. This step was repeated 2 times, and then contents of the channels were emptied. Dispense 1.2 ml of anti IgG conjugate (R3) into each channel. Place the tray on the rocking platform. Incubate for 60 min \pm 5 min at 18-25°C, then washing step. Distribute 1.2 ml of NBT/BCIP substrate (R5) into each of the channels. Place on the rocking platform and protect from direct light. Incubate for 60min \pm 5 min at 18-25°C.

Stop the reaction by aspirating substrate with a Pasteur pipette or by turning the incubation tub over and dispensing 2 ml of distilled water in the channels. Repeat this last washing step one more time. The color of the strips will naturally lighten while drying. Interpretation must only be performed after drying is complete.

A positive sample can present numerous bands between 15 and 200 kilodaltons (kDa). The low molecular weight bands of 24 - 35 kDa for each of the tested samples are characteristic and generally easily found. Two groups of bands of high molecular weight (HMW) in the 70- 90 kDa and 100-200 kDa range are

not specific to toxocariasis (possible cross reaction with other helminthiases).

5. Statistical analysis: The collected data were tabulated and statistically analyzed using statistical package for social science (SPSS) version 21 (SPSS Inc. USA). Quantitative data were represented as the mean and standard deviation (SD) and for comparison between two groups; the independent samples student (t) test was used. For interpretation of results, p value ≤ 0.05 was considered significant.

RESULTS

The study included 144 cases divided into two groups. The group (1) is the asthmatic group and included 72 children (42 males and 30 females) with confirmed asthmatic bronchitis and the group (2) is the control group (Non asthmatic) and included 72 children (39 males and 33 females).

Sixteen (22.2%) of asthmatic group were seropositive for anti-*Toxocara* IgG using immunoblot

assay technique, while in control group it was 6.9% (n=5). The results show higher *Toxocara* seropositivity rates in the asthmatic children but there was a significant difference between both patient and control groups in relation to seroprevalence of *Toxocara* (P<0.05), as shown in (Table 1). Regarding the sociodemographic data, there was a significant association between the seroprevalence of *Toxocara* and contact with pets, geophagia and residence (P<0.05). However, sex difference and socioeconomic level were found to be non-significant (P>0.05), as shown in (Table 2). Concerning the clinical manifestations, significant association was detected between the seroprevalence of *Toxocara* and respiratory manifestations, hepatomegaly and splenomegaly (P<0.05), as shown in (Table 3). As regards eosinophilia there was no significance difference in seropositive toxocariasis and eosinophilia (P>0.05), as shown in (Table 4).

Table 1: Seroprevalence of *Toxocara* between both asthmatic and control groups using immunoblot assay for anti *Toxocara* IgG.

| | Asthmatic group | | Control group | | Chi-Square | |
|-----------------|-----------------|-------|---------------|-------|----------------|---------|
| | N | % | N | % | X ² | P-value |
| Positive | 16 | 22.2 | 5 | 6.9 | 6.746 | 0.0078 |
| Negative | 56 | 78.8 | 67 | 93.1 | | |
| Total | 72 | 100.0 | 72 | 100.0 | | |

Table 2: Comparison of anti *Toxocara* IgG positive cases between asthmatic and control group as regards sociodemographic data.

| | | Positive IgG Asthmatic Group (N=16) | | Positive IgG Control Group (N=5) | | Chi-Square | |
|----------------------------|-----------------|-------------------------------------|-------|----------------------------------|-------|----------------|---------|
| | | N | (%) | N | (%) | X ² | P-value |
| Gender | Male | 12 | 75 | 3 | 53.57 | 0.42 | 0.517 |
| | Female | 4 | 25 | 2 | 45.42 | | |
| Socioeconomic level | High | 3 | 18.75 | 1 | 20 | .1706 | 0.9182 |
| | Moderate | 5 | 31.25 | 2 | 40 | | |
| | Low | 8 | 50 | 2 | 40 | | |
| Contact with pets | Yes | 13 | 81.3 | 1 | 20 | 5.431 | 0.011 |
| | No | 3 | 18.7 | 4 | 80 | | |
| Geophagia | Yes | 15 | 93.75 | 2 | 40 | 7.138 | 0.007 |
| | No | 1 | 6.25 | 3 | 60 | | |
| Residence | Rural | 15 | 93.75 | 2 | 40 | 7.137 | 0.008 |
| | Urban | 1 | 6.25 | 3 | 60 | | |

Table 3 - Symptoms and signs associated with toxocariasis cases in asthmatic group and control group.

| Symptoms and Signs | Asthmatic Group Positive IgG Cases (N=16) | Control Group Positive IgG Cases (N=5) | Chi-Square |
|--------------------|---|--|------------|
|--------------------|---|--|------------|

| | | N | (%) | N | (%) | X ² | P-value |
|----------------------------|-------------|----|-------|---|-------|----------------|---------|
| Chronic cough | present | 16 | 100 | 0 | 0 | 16.36 | 0.00006 |
| | Not present | 0 | 0 | 5 | 100.0 | | |
| Wheezing | present | 12 | 75 | 0 | 0 | 6.865 | 0.027 |
| | Not present | 4 | 25 | 5 | 100.0 | | |
| Dyspnea | present | 10 | 62.5 | 0 | 0 | 4.427 | 0.035 |
| | Not present | 6 | 37.5 | 5 | 100.0 | | |
| Neurological manifestation | present | 6 | 18.75 | 0 | 0 | 0.198 | 0.910 |
| | Not present | 13 | 81.25 | 5 | 100.0 | | |
| Skin manifestation | present | 7 | 43.75 | 2 | 40 | 0.0219 | 0.882 |
| | Not present | 9 | 56.25 | 3 | 60 | | |
| Liver involvement | Normal | 6 | 37.5 | 5 | 100.0 | 4.427 | 0.035 |
| | Enlarged | 10 | 62.5 | 0 | 0 | | |
| Spleen involvement | Normal | 5 | 31.25 | 5 | 100.0 | 5.540 | 0.0185 |
| | Palpable | 11 | 68.75 | 0 | 0 | | |
| Lymph node involvement | Normal | 3 | 18.75 | 2 | 40 | 0.948 | 0.330 |
| | Palpable | 13 | 81.25 | 3 | 60 | | |

Table (4): Comparison of cases with eosinophilia regarding anti- *Toxocara* spp. IgG immunoblot in asthmatic and control groups.

| | Patients with eosinophilia in asthmatic Group (N=44) | Patients with eosinophilia in control group (N=17) | Chi-Square | |
|--|--|--|----------------|---------|
| | | | X ² | P Value |
| Anti- <i>Toxocara</i> Spp. IgG immunoblot positive Cases | 16 (36.36%) | 5 (29.41%) | 0.0262 | 0.608 |
| Anti- <i>Toxocara</i> Spp. IgG immunoblot negative Cases | 28 (63.63%) | 12 (70.58%) | | |

are more likely to wheeze in response to larval invasion (7).

DISCUSSION

Toxocariasis was reported as one of the widely world distributed geoparasites (14) where about 21% of the population was infected with one or more intestinal geohelminths. Also bronchial asthma was increased in the industrialized countries (15) where the incidence reached 20.8% corresponded to those recorded in Latin American countries and in some developed countries worldwide (16). The infection with toxocariasis was considered as a potential risk factor for bronchial

asthma (17) where some studies showed positive association in human (18,19). Migration of *Toxocara* larvae in the lung cause reactive respiratory changes and infected individuals

Regarding the immunoblot IgG analysis of the studied groups, 22.2% (n= 16) of asthmatic patients were IgG seropositive for toxocariasis while 6.9 % (n=5) of healthy children were IgG seropositive. There was a significant correlation between toxocariasis and asthma. These results were agreed with that obtained by **Badawey** (14) in Zagazig, Sharkia, Egypt who reported that 17%% of toxocariasis was present in asthmatic patients and 10% in controls. Also, **El-Tantawy** (20) in Mansoura, Dakahlia Egypt reported 42%% of toxocariasis was found in asthmatic patients and 8% in controls and **Kanobana** (21) reported that antibodies of *Toxocara* spp. were found in 40.1% of the children.

From the demographic data of the studied groups, male to female ratio was approximately 3:1 in asthmatic group and 1.5:1 in control group. Among the 16 asthmatic patients positive for toxocariasis, there was a

percentage in the male gender of 75% (n = 12) and in the female of 25% (n = 4). And among 5 healthy children in control group diagnosed with toxocariasis, there was a percentage in the male gender of 60% (n = 3) and in the female of 40% (n = 2). There was no significant difference between cases and control group as regard the sex. These results were agreed with **Badawey**⁽¹⁴⁾ in Zagazig, Sharkia, Egypt who reported that no significant difference between toxocariasis and sexes. **Nyan**⁽²²⁾ in Banjul reported no significant difference among sexes regarding intestinal helminthic infections. However, **Silva**⁽²³⁾ found a two-fold increase in risk of toxocariasis in boys compared to girls. This might be because boys always play outdoors⁽²⁴⁾ and have more exposure to contaminated environments⁽²⁵⁾.

The present study found no significant difference between toxocariasis and socioeconomic level. But, **Souza**⁽²⁶⁾ in Salvador recorded a positivity rate of 59.9% with a higher incidence among lower social classes. Also, **Alvarado-Esqui**⁽²⁷⁾ reported that socioeconomic status was an important factor in positive rate of toxocariasis. However, **Silva**⁽²³⁾ did not accept such an effect using family income and maternal schooling as a monitor for social classes.

In the present study there was a significant relation between *Toxocara* infection and residence where high rate of toxocariasis 93.75% (n=15) was in rural area versus 6.25% (n= 1) in urban one among asthmatic group and 40% (n= 2) in rural area versus 60% (n= 3) in urban one among control group. These results were agreed with **Badawey**⁽¹⁴⁾ in Zagazig, Sharkia Egypt who reported that toxocariasis was more common in rural 86% versus 14% in urban one. While **Mendonca**⁽²⁸⁾ in Brazil reported high rates of toxocariasis (63.6%) in elementary school children in urban and semi-rural areas. But **Nyan**⁽²²⁾ in Banjul reported that toxocariasis was more common in rural 17% versus 8.2% in urban one.

In the present study, there was a significant correlation between toxocariasis and pet contact. This study was reported that 13 cases (81.3%) were in contact with pets among asthmatic group (n=16) compared to 1 case (20%) among control group (n=5). This in accordance with the previous results of **Badawey**⁽¹⁴⁾ who found that a significant relation between toxocariasis and pet contract. Many studies suggested that dog contact was the main risk factor of *T. canis*, as a direct source of infection, but cat exposure was less frequently as cats cover feces under sand⁽²⁹⁾.

The present study found highly significant difference between toxocariasis and geophagia where 15 cases (93.75%) were in contact with soil among asthmatic group (n=16) compared to 2 cases (40%)

among control group (n=5) which coincided with **El-Tantawy**⁽³⁰⁾ who Found that eating soil (pica) was a risk factor for toxocariasis.

In the present work, it was observed that the reported clinical signs (respiratory manifestations, hepatosplenomegaly) were suggestive signs of toxocariasis and have a positive correlation. The reported clinical manifestations among asthmatic group (n=16), 16 cases (100%) were complaining of chronic cough, 12 cases (75%) were associated with wheezing, 10 cases (62.5%) were associated with dyspnea, 3 cases (18.75 %) were suffering from neurological manifestation, 7 cases (43.75 %) were associated with skin manifestation, 10 cases (62.5%) were associated with enlarged liver, while 11 cases (68.75 %) were associated with palpable spleen and 13 cases (81.75 %) were associated with palpable lymph node. Comparing to positive control group cases (n=5) ,2 cases (40%) were associated with skin manifestations and 3 cases (60%) were associated with palpable lymph node.

There was significant relation between *Toxocara* seropositivity and respiratory symptoms as well as hepatosplenomegaly. This is in accordance with the previous results of **Bahnea**⁽³¹⁾ who find that children with seroprevalence for *Toxocara* have a significant relation to enlarged liver and spleen. Also, **Gueglic**⁽³²⁾ reported that the most frequent clinical respiratory manifestation was pulmonary symptoms and the most frequent digestive symptoms were abdominal pain and hepatosplenomegaly in seropositive adults.

In the present work there was no significant difference between toxocariasis and eosinophilia level between asthmatic and control groups. This result was agreed with previous results of **Badawey**⁽¹⁴⁾ who founded that no significant difference between toxocariasis and eosinophilia level in asthmatic patients, but a highly significant difference between *Toxocara* spp. infection and eosinophilia among controls.

Our study revealed that 16/44 (36.36%) patients had eosinophilia were seropositive for antitoxocara IgG among asthmatic patients compared to 5/17 (29.41%) among control group. **Gueglic**⁽³³⁾ tested 39 eosinophilic patients and found positive results in 71.8% of toxocariasis cases Also, they found that eosinophilic patients were at 149 times greater risk of being *Toxocara* seropositive compared to patients who had no eosinophilia while **El-Shazly**⁽³⁴⁾ found that positive results in 29% of eosinophilic cases.

Conclusion

Rising knowledge of toxocariasis will enable pediatricians to put in mind it as a cause of asthmatic bronchitis in their patients, initiate appropriate

treatment, and educate patients and their parents how to avoid infection.

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