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Seroepidemiological Evaluation of Toxocariasis in Egyptian Children Suffering from Recurrent Urticaria

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ABSTRACT

Background: human toxocariasis is one of the most common zoonotic helminthiases reported all over the world. Large spectrum of clinical diseases due to toxocariasis including cutaneous diseases particularly urticaria has been identified by recent diagnostic laboratory tests. **Objective:** it was to evaluate the *Toxocara* seroprevalence in children diagnosed with chronic urticaria (CU) and to identify its possible relationship with epidemiological, laboratory and clinical aspects, through a case–control study presented at the Dermatology Outpatient Clinic of Al-Azhar University Hospital in New Damietta City. **Patients and Methods:** seventy children diagnosed with CU and seventy healthy controls were included in the study. Sociodemographic risk factors for toxocariasis were analyzed based on a questionnaire collected from the children's parents or guardians, then all of the children were subjected to thorough clinical examination, stool examination to exclude other parasites and blood samples were collected and tested for eosinophilic count by CBC as well as estimation of anti-*Toxocara* IgG antibodies by means of western blot (WB) test. **Results:** *Toxocara* IgG was positive in 18.5% (n=13). It was significantly higher than among the cross-matched 70 healthy controls (4.2%). This study confirmed the possibility of significant association between the seroprevalence of *Toxocara* and possible socioepidemiological factors as contact with pets or soil, geophagia as well as state of house. Regarding clinical manifestations and laboratory investigations, bronchial asthma, rhinitis and conjunctivitis were found to be significantly associated with *Toxocara* seroprevalence.

Conclusion: *Toxocara* infection should be considered as an important cause of recurrent urticaria among children population in developing countries.

Keywords: Chronic urticaria, Toxocariasis, Children, IgG, Seroprevalence.

INTRODUCTION

Toxocariasis is a helminthic zoonosis caused by the round worm *Toxocaracanis* that infects dogs and less frequently by *Toxocara cati*, which infects cats. Children may be infected by the ingestion of *Toxocara* spp. eggs through hand contamination, soil ingestion, direct contact with dogs and cats, and with objects contaminated with infective eggs of these parasites[(1)].

When humans who are incompatible hosts accidentally ingest infective eggs and larvae, they become free in the intestine and pass through the intestinal epithelium to reach the blood vessels, where they can migrate to various visceral organs and tissues of the body without developing into the adult form[(2)].

Toxocariasis was described in three clinical forms: visceral larva migrans (VLM), ocular larva migrans (OLM) and subclinical or covert toxocariasis**.** The spectrum of the clinical manifestations varies widely from symptomless cases to systemic infections[(2)]**.** The clinical syndrome of VLM may be acute or subacute, presented with hepatomegaly, splenomegaly, gastrointestinal manifestations, cutaneous manifestations, central nervous system involvement, pulmonary involvement, pyrexia and eosinophilia[(3)]**.**

Urticaria is a pathological condition characterized by itchy erythematous, edematous blisters with temporary rashes. It is a common disease that is seen in 15-25% of people in at least one part of their lifetime. Urticaria is classified as acute or chronic according to its duration. Clinical findings above six or more weeks are defined as CU. About 75% of the urticarial cases are idiopathic. Drugs, food and helminthic infections seem to be the etiological factors. It is reported that urticarial lesions are observed in approximately 80% of parasitosis cases[(4)]**.**

According to cutaneous manifestations, toxocariasis may be associated with rash, pruritus and chronic urticaria**.** According to some researches, hypereosinophilia is the main reason of the skin symptoms which is explained by the release of cutaneous chemotactic factor with the effect of eosinophils. Another clue is the histamine release caused by the proteinase activity of *Toxocara* excretory-secretory (TES) antigens[(5)]**.**

Seroprevalence of toxocariasis has been reported to fluctuate between 2 and 92.8% according to geographical region and age group[(6)]**.** Studies

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investigated toxocariasis on recurrent urticarial patients are limited. But a possible relation between antibodies against *Toxocara*, CU and eczema has been proposed in previous studies but could not clearly been demonstrated[7].

Toxocariasis in human is mostly unknown either to health professionals and/or to the most of Egyptian population because it is still a poorly diagnosed disease[8]. It is difficult to be diagnosed by the direct techniques, thus the confirmatory diagnosis of toxocariasis depends greatly on immunological tests. The enzyme-linked immunosorbent assay (ELISA) is the method used for diagnostic and for epidemiological studies depending on the detection of IgG, the immunoglobulin which is reactive to*Toxocara* excretory-secretory antigens. The sensitivity and specificity of this method varies according to the antigen used [9].

Western blotting (WB) 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 test has been successfully adapted for the confirmatory serodiagnosis of various parasitic diseases, including schistosomiasis, hydatidosis, cysticercosis, taeniasis, fascioliasis and strongyloidosis[10]. The best choice for sero-diagnosis of the different clinical forms of toxocariasis, visceral larva migrans (VLM) or covert toxocariasis is the initial use of TES-ELISA after which any positive result should subsequently be tested by Western blotting[11].

Aim of the study: This study aims to investigate the *Toxocara* seropositivity in urticarial children with WB test, in Damietta city, Egypt and to evaluate possible risk factors associated with the infection.

PATIENTS and METHODS

Study area and population: This case-control study was conducted from March 2017 to December 2017, on 70 children aged between 4 and 13 years suffered from recurrent urticaria and 70 healthy cross-matched children as controls. All attended to the outpatient clinic of Dermatology Department at Al-Azhar University Hospital in New Damietta, Egypt. Children were enrolled in the study after obtaining verbal informed consent from their parents or guardians.

Ethical approval:

The study protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Al-Azhar University. Socioepidemiological data were collected from the children's parents or guardians using a questionnaire to assess the risk factors associated with *Toxocara* infection as age, sex, residence, pets and soil contact, geophagia, parent's education, family income and house type.All the children were subjected to detailed clinical examination.

Sample collection:

Four ml blood samples were collected from all the participant children, placed in 2 tubes, one containing anticoagulant for complete blood count (CBC) with differential leukocytes count especially looking for those with eosinophilia levels above 400/ mm^3[2] and the second tube was centrifuged. Collected serum was stored at −20°C until used.

Fecal samples were also collected from all the children for three consecutive days and were macroscopically examined for color, consistency, mucus and presence of worms or parts of them. Stained direct smears and formalin-ethyl acetate concentration as well as flotation slides were examined microscopically by low and high powers to detect helminthic ova, larvae, trophozoites and cysts. Positive cases with any of these parasites were excluded directly from the present research to avoid cross reaction possibility.

Serological investigation:

The serum samples were tested for anti-*Toxocara* IgG using *Toxocara* Western Blot IgG (LDBIO Diagnostic, 19A rue Louis Loucher, Lyon, France) which detect antibodies against the excretory/secretory (ES) antigens of the *Toxocara* larvae. *Toxocara* (ES) antigens have been resolved by electrophoresis into bands and transferred by electro blotting on a nitrocellulose membrane (Western Blot procedure). The antigen bearing membrane has been cut into ready to use numbered strips. Each specimen tested was incubated separately with a membrane strip. If specific anti-*Toxocara* antibodies were present in the sample, they had bound to the antigens on the strip. The first wash step removed any unbound serum components. Thenthe strip was incubated with an alkaline phosphatase-anti human IgG conjugate. After the second wash step, a substrate of (NBT/BCIP) was added for detection of the whole complex which precipitated as a dark blue-purple color. The color development was stopped by rinsing the strips with distilled water. S

pecific IgG anti-*Toxocara*, if present in the sample, has appeared on the strip as violet coloured bands. The strips were then dried and their coloration remains stable for many years once stored away from direct light. The presence on the strip of 5 bands located between 24 and 35 kDa Molecular Weight (MW) was indicative of the presence of specific anti-*Toxocara* IgG in the sample.

Statistical analysis

All data were introduced in an Excel spreadsheet and statistical analysis was carried out using the SPSS statistical package version 19. Fisher's exact test was performed to confirm the difference between groups. The level of significance selected was p value < 0.05.

RESULTS

The study included 70 children having CU and 70 healthy controls. Western blotting showed that 18.5% (n=13) of CU children were seropositive for *Toxocara*, while in healthy controls it was 4.2% (n=3). The results show higher *Toxocara* seropositivity rates in the urticarial 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 socioepidemiologic factors, there was a significant correlation between the seroprevalence of *Toxocara* and contact with pets or soil, geophagia and state of house (P<0.05). However, sex difference, rural residence, family income and education found to be non-significant (P>0.05), as shown in **(**Table **2)**.

Concerning the clinical manifestations and laboratory investigations, a significant association was detected between the seroprevalence of *Toxocara* and bronchial asthma, rhinitis and conjunctivitis (P<0.05), as shown in **(**Table **3)**.

Table (1): Seroprevalence of *Toxocara* between both urticarial and control Groups

	Urticarial group		Control group		Chi-Square	
	N	%	N	%	X^2	P-value
Positive	13	18.5	3	4.2	7.056	0.0078
Negative	57	81.5	67	95.8		
Total	70	100.0	70	100.0		

Table (2): Comparison of anti-*Toxocara* spp. IgG positivity between urticarial and control groups according to socioepidemiologic variables

		Urticarial group		Control group		Chi-Square	
		N	%	N	%	X^2	P-value
Gender	**Male**	9	69	2	66.6	0.0075	0.931
	Female	4	31	1	33.4		
Contact with pets	**Yes**	12	92.3	1	33.4	5.564	0.018
	No	1	7.7	2	66.6		
Contact with soil	**Yes**	13	100	1	33.4	6.48	0.010
	No	0	0	2	66.6		
Geophagia	**Yes**	12	92.3	1	33.4	5.564	0.018
	No	1	7.7	2	66.6		
Residence	**Rural**	8	61.5	2	66.6	0.027	0.868
	Urban	5	38.5	1	33.4		
Educational level	**Educated**	6	46.1	1	33.4	0.162	0.686
	Ignorant	7	53.9	2	66.6		
Family income	**Satisfactory**	6	46.1	1	33.4	0.162	0.686
	Not satisfactory	7	53.9	2	66.6		
House state	**Good**	1	7.7	2	66.6	5.564	0.01
	Poor	12	92.3	1	33.4		

Table (3): Comparison of anti-*Toxocara* spp. IgG positivity between urticarial and control groups according to clinical and laboratory variables

		Urticarial group		Control group		Chi-Square	
		N	%	N	%	X^2	P-value
Pyrexia of unknown origin	Yes	1	7.6	0	0	0.553	0.456
	No	12	92.4	3	100		
Hepatomegaly or hepato-splenomegaly	Yes	2	15.3	0	0	0.055	0.813
	No	11	84.7	3	100		
Lymphadenopathy	Yes	1	7.6	1	33.4	1.465	0.226
	No	12	92.4	2	66.6		
Neurological disorders	Yes	1	7.6	0	0	0.553	0.456
	No	12	92.4	3	100		
Bronchial asthma	Yes	12	92.4	1	33.4	5.564	0.0183
	No	1	7.6	2	66.6		
Gastrointestinal troubles	Yes	2	15.3	1	33.4	0.515	0.472
	No	11	84.7	2	66.6		
Rhinitis	Yes	12	92.4	1	33.4	5.564	0.0183
	No	1	7.6	2	66.6		
Dermatitis	Yes	7	53.8	0	0	0.162	0.686
	No	6	46.2	3	100		
Conjunctivitis	Yes	10	76.9	0	0	4.923	0.026
	No	3	23.1	3	100		
Anemia	Yes	2	15.3	1	33.4	0.515	0.472
	No	11	84.7	2	66.6		
Eosinophilia	>400	11	84.6	3	100	0.055	0.813
	<400	2	16	0	0		

DISCUSSION

Zoonotic toxocariasis was reported as one of the widely distributed geoparasites in Egypt[12]. *Toxocara* eggs may hatch in the human small intestine, but larvae cannot develop into adults in humans. Larvae then penetrate the intestinal wall and migrate to multiple organs via the blood stream;diverse clinical features may develop according to the involved organ[2]. This happens mainly among children when they ingest soil while playing or eating[13]. Skin symptoms are usually observed as chronic itching, chronic urticaria and eczema types[5]. Demographic and socioeconomical factors may lead to increase in *Toxocara*seroprevalence[7].

This case-control study aimed to evaluate the *Toxocara* according to seroepidemiological factors in the locality of (Damietta, Egypt), among urticarial children (as they are more prone to infection) to observe a probable association between urticaria and positive serology for *Toxocara* by WB. The categorization of all participantchildren was determined by means of a questionnaire.

Studies exploring toxocariasis on chronic urticarial patients are limited and possible relation between antibodies against *Toxocara* and CU and eczema has been proposed in previous studies but could not clearly been demonstrated[4, 7].

The present findings showed that *Toxocara* seropositivity was 18.5% (13/70) in urticarial children, demonstrated higher *Toxocara* seropositivity rates which approximately agreed with previous observations by **Fialho *et al.*[7]** which was18.7% and **Humbert *et al.*[15]** which was 19.5%**,** but less than that obtained by **Demirci *et al.*[4]** which was 29% and **Wolfrom *et al.*[14]** which was 65%. On the other hand, **Grama *et al.*[16]** found no association between toxocariasis and urticaria in atopic children.

The present work results demonstrated that there was a significant difference between both urticarial and healthy control groups in relation to seroprevalence of *Toxocara* which was 18.5% (13/70) in urticarial children and 4.2% (3/70) in controls, that approximately agreed with **Wolfrom *et al.*[14]** and **Humbert *et al.*[15],**

but **Demirci *et al*.**[4] found that it was non-significant. **Grama *et al*.**[16] did not observe any *T. canis* IgG positivity in healthy controls.

The present study showed no significant association between the seroprevalence of *Toxocara* and sex difference, but reported a predominance of *T. canis* in boys (69%) than girls (31%), this might be due to thatboys always play outdoors and more exposed to contaminated environments[17]**. Ajayi *et al*.**[18] and **Nihal *et al*.**[19] reported that the gender not seemed to be an important factor related to a positive serology for *Toxocara*. Also, **Nyan *et al*.**[20] in Banjul reported no significant difference between sexes regarding infection by intestinal helminthes. **Silva *et al*.**[21] found a double increase in risk of toxocariasis in boys compared to girls. **Iddawela*et al*.**[22] found that the rate of seropositivity for *Toxocara* was approximately equal in both males and females.

The present work reported that contact with pets was significantly associated with the seroprevalence of *Toxocara* infection which was 92.4% in seropositive urticarial children and 33.4% in seropositive controls. This result was agreed with that obtained by **Wolfrom *et al*.**[14], **Figueiredo *et al*.**[2] and **Badawy*et al*.**[23]**.** Many studies suggested that contact with dogs was the main risk factor of *T. canis*, as a direct source of infection, but contact with cats was less frequently as the cats cover feces under soil[24]**.** On the other hand, the present results not agreed with that obtained by **Mattia *et al*.**[25]**, El Tantawey *et al*.**[26] and **Gramaa *et al*.**[16] who found no significant association between *Toxocara* infection and pets contact.

Thisstudy proved that the association between contact with soil and seroprevalence of *Toxocara* was significant, which was 100% in seropositive cases and 33.4% in seropositive controls. These results were agreed with that obtained by **Figueiredo *et al*.**[2]**, Etewa *et al*.**[12] and **Badawy *et al*.**[23]**,** but not agreed with that obtained by **Gramaa*et al*.**[16]**.**

In the present work there was significant association between the seroprevalence of *Toxocara* and geophagia, which was 92.4% in seropositive cases and 33.4% in seropositive controls. This is in accordance with the previous results of **Buijs**[27] and **Figueiredo *et al*.**[2]**.** However **Gramaa *et al*.**[16] found no association between *Toxocara* infection and geophagia.

The present study showed significant association between the seroprevalence of *Toxocara* and poor house state which was 92.4% in seropositive cases and 33.4% in seronegative ones. The presences of sand, earth or cement around the home are characteristic factors that may be appropriate for infection by human toxocariasis. Furthermore, presence of dogs and cats may increase the risk of infection.Also, Lack of hygiene in poor houses is considered to be an increased risk factor for infection. This was agreed with results obtained by **Etewa *et al*.**[12]**,** but not agreed with **Gramaa *et al*.**[16] and **Cadore *et al*.**[28]**.**

In the present work there was no significant association between seroprevalence of *Toxocara* and rural residence, which was 61.5% in seropositive cases and 66.6% in seropositive controls. In the chosen locality, infection can occur in both urban and rural areas due to contact with contaminated ground by *Toxocara* ova from infected stray dogs or contact with domestic pets. This agreed with results obtained by **El Tantawey *et al*.**[25] and **Badawy *et al*.**[23]**. Nyan *et al*.**[20] and **Mendonca *et al*.**[29] reported that toxocariasis was more common in urban versus rural one. **Alavi and Sefidgaran**[30] in Iran found similar *Toxocara* seropositive between rural and urban. **Nihal *et al*.**[19] and **Antonios *et al*.**[31] found high rates of toxocariasis in rural than in urban areas.

The present study results showed no significant association between the seroprevalence of *Toxocara* and educational level of the child's parents probably due to the fact that this population lives in the same environment and is subjected to the same infections. This result agreed with that obtained by **Figueiredo *et al*.**[2] and **Etewa *et al*.**[12] but not agreed with **Badawy *et al*.**[23]**.**

In The present study there was no significant association between the seroprevalence of *Toxocara* and family income. This result agreed with that obtained by **Etewa *et al*.**[12]**,** but not agreed with that obtained by **Souza *et al*.**[32]**, Alvarado-Esquivel**[33] and **Badawy *et al*.**[23]**.**

We have observed that there was significant association between the seroprevalence of *Toxocara* and associated bronchial asthma, rhinitis and conjunctivitis, but we did not find any association between toxocariasis and atopic dermatitis. **Buijs*et al*.**[34] had studied children with toxocariasis and found significant association with allergic disorders, such as asthma and also with IgE specific to inhaled allergens. They reported that parasitic infections including *Toxocara* infection, causes a nonspecific stimulation of quiescent allergic disorders in atopic children.

The present study reported no significant association between the seroprevalence of *Toxocara* and eosinophilia which was 84.6% in seropositive cases and 100% in seropositive controls. This result was agreed with that obtained by **Gramaa *et al*.**[16]**, Iddawela *et al*.**[22] and **Badawy *et al*.**[23], but not agreed with that

obtained by **Figueiredo *et al*.**[(2)] and **El Tantawey *et al*.**[(26)].

CONCLUSION

The results pointed to a possible role of *Toxocara* infection in children suffering from recurrent urticaria, especially those exposed to an increased risk of environmental exposure to toxocariasis as contact with pets, contact with soil and geophagia, and also in children with bronchial asthma, rhinitis and conjunctivitis. Physicians especially dermatologists should pay attention to incorporate toxocariasis in the differential diagnosis of chronic urticaria.

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