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Seropositivity and risk factors of *Toxocara canis* infection in adult asthmatic patients

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Abstract. This cross-sectional study involving 86 adult asthmatic patients aimed to determine the relationship between *Toxocara* seropositivity and severity of asthma in adult asthmatics and investigate the risk factors for *Toxocara* infection. In all cases, *T. canis* IgG level was measured using an anti-*Toxocara* IgG enzyme-linked immunosorbent assay kit. Total serum IgE and eosinophil count were also determined. The anti-*Toxocara* IgG seropositivity was 68.6% among asthmatic patients. There were no statistically significant associations between *Toxocara* seroprevalence and other risk factors, clinical symptoms of asthma and high level of total serum IgE and eosinophilia. Pet ownership could be an important risk factor for Toxocariasis. Having a pet at home and wheezing were significantly associated with *Toxocara* seropositivity in adult asthmatic patients.

INTRODUCTION

Toxocariasis is a human parasitic disease caused by *Toxocara canis* and *Toxocara cati*, roundworms of dogs and cats respectively. Toxocariasis is common in developing countries, especially in tropical regions and among poor population. Dogs are definitive hosts and can be infected by *T. canis* through ingestion of embryonated eggs or trans-mammary or trans-placental transmission of larvae. Human can be infected via ingestion of embryonated *T. canis* eggs as well. The major means of transmission of infection in human is ingestion of embryonated eggs excreted by dogs' feces found in contaminated soil or uncooked meat or unwashed vegetables or fruits, or through accidental consumption of raw/undercook paratenic host meat containing infective larvae (Yoshida *et al.*, 2016). Although human is a paratenic host for *T. canis*, the parasite cannot be developed into an adult worm. Few larvae are required to cause disease in humans. Most infections by *T. canis* is asymptomatic such as covert-toxocariasis or (CT) and the morbidity of this syndrome depends on the parasitic burden and host immune system bias. Immunological detection assays for toxocariasis detection is based on native or recombinant TES antigens or either glycan antigens or deglycosylated TES antigens and antibodies like total IgG, IgG subclasses or IgM (Ma *et al.*, 2014).

Epidemiological studies suggest that infections with *Toxocara* can cause allergic reaction such as asthma with symptoms like wheezing, coughs, mucus hyper-secretion and bronchial hyper-reactivity (Pinelli *et al.*, 2012). Hygiene hypothesis suggest that helminth infections such as *Ascaris lumbricoides*, *Schistosoma mansoni* and *Trichuris trichiura* can protect human body from allergic diseases, whereas *Toxocara* can develop this immunopathology in children in particular.

Although few studies suggested no co-relation between *Toxocara* infection and asthma, no studies have been reported on an inversion association. Hygiene hypothesis

suggested that because of lack of infection in children there is weaker Th1 response which allows increasing of Th2 response toward environmental allergens. Interaction between helminth infection and allergy involved Treg cells (Pinelli *et al*., 2012).

Toxocara infection is associated with a polarized $CD4^+$ Th2 response with high level of IgE and eosinophilia which are mediated by HLA class II molecules. There is association between HLA class II and pathological severity. Besides, it has been suggested that $Foxp3^+$ $CD4^+$ $CD25^+$ – expressing T regulatory (Treg) cells can regulate the immunopathology of granulomas in experimental toxocaral granulomatous hepatitis and can increase the level of TGF-β1 expression level which is important for local survival and function of Treg during invasion of *T. canis* in intestine, live, muscle and brain (Fan *et al*., 2013).

Toxocariasis is associated with elevated levels of specific IgE against aeroallergens (sIgE), serum total IgE, eosinophilia, increased skin sensitivity to aeroallergens, atopic asthma and decreased lung function. *Toxocara* can cause allergy reactions by inducing Th2 response. Glycan antigens, which are located on glycoproteins of *Toxocara* larvae, have an important role in induction of this lymphocyte that consequently increases the production of IL-4 and switches B cells for IgE synthesis. Further, Th2-type lymphocyte increases production of IL-5, which increases the total numbers of eosinophils, macrophages and mast cells. Eosinophilia causes smooth muscle layers to thicken and consequently narrows down the airways. Besides, eosinophils can produce free radicals that damages trachea and causes pulmonary inflammation. Asthma is the most wide spread pulmonary disease in the world and its prevalence is 0–30% in children and around 10% in adults. The clinical symptoms of asthma include wheezing, cough, mucus hyper-secretion and bronchial hyper-reactivity (Mosayebi *et al*., 2016; Pinelli *et al*., 2012; Qualizza *et al*., 2009). Atopy is the most important risk factor of asthma because about half of asthma cases are atopic, while epidemiological studies suggest that *Toxocara* plays a major role in pathogenesis of atopy (Pinelli *et al*., 2012). Experimental studies suggested the association and co-relation of toxocariasis and development of allergic disease such as asthma (Li *et al.*, 2014). It has been identified by Li *et al.* (2014) that there is association between toxocariasis and lung function. Toxocariasis is a common helminth infection, therefore detection and estimation of *Toxocara* infection and asthma is necessary (Li *et al*., 2014). The aim of this study was to determine the relationship between *Toxocara* seropositivity in adult asthmatic patients, and to investigate the risk factors of Toxocara infection.

MATERIAL AND METHODS

Study population

A cohort of 86 adult asthmatic patients aged between 19 and 92 years participated in this study between August 2016 and February 2017 at University Malaya Medical Centre. The study protocol was accepted by Ethics Committee of Faculty of Medicine, University of Malaya (MEDIC.NO: 20161-2042). Informed consent forms were filled out by all patients. Risk factors shown in Table 1 were used, based on the published papers and ISSAC questionnaire.

Measurement of total IgE ELISA kit using adult asthmatic serum samples

Eighty-six asthmatic adults' sera were used in a commercial total *Toxocara* IgE Enzyme Immunoassay Test Kit (BioCheck, Inc, CA, USA). Twenty microliters of standard specimens and controls were dispensed into appropriate wells in ELISA microtiter plate. One hundred microliter of blocking buffer were added to each well. The plate was thoroughly mixed on a plate shaker for 30 seconds. The plate was incubated at room temperature (18 to 25°C) for 30 minutes. The plate was washed and rinsed 5 times in distilled or deionized water. After the last rinse, the wells were slapped out on a clean absorbent towel to remove excess wash buffer. Hundred and fifty microliters of

enzyme conjugate reagent were added to all the wells, gently mixed for 10 seconds. The plate was incubated at room temperature for 30 minutes. The plate was washed and rinsed 5 times in distilled or deionized water. After the last rinse, the wells were slapped out on a clean absorbent towel to remove excess water. One hundred microliters of TMB reagent were added to each well and gently mixed for 10 seconds. The plate was incubated at room temperature for 20 minutes. One hundred microliters of reaction stopping solution were added to each well and were gently mixed for 30 seconds. The optical density (OD) was read at 450 nm with microtiter plate reader within 15 minutes.

Measurement of *Toxocara* IgG ELISA using adult asthmatic serum samples

Eighty-six adult asthmatic patients' sera were used in a *Toxocara* IgG Enzyme Immunoassay Test Kit (AccuDiag™ Toxocara IgG ELISA Kit; CA, USA). Patients' sera were diluted (1:64 dilution). One hundred microliters of negative control, positive control and diluted sera were added to the remaining wells. The ELISA plate was incubated at room temperature for 10 minutes. The wells were washed and rinsed three times using the wash buffer. After the last rinse, the wells were slapped out on a clean absorbent towel to remove excess wash buffer. One hundred microliters of enzyme conjugate were added to each well. The ELISA plate was incubated at room temperature for 5 minutes and then washed and rinsed three times. After the last rinse, the wells were slapped out on a clean absorbent towel to remove excess wash buffer. One hundred microliters of chromogen (TMB) were added. The ELISA plate was incubated at room temperature for 5 minutes. One hundred microliters of reaction stopping solution were added to each well. The ELISA plate was tapped gently on the side of the strip holder with index finger for approximately 15 seconds to mix wells. The absorbance of samples at wavelength of 450 nm was measured using a microplate reader. Absorbance read greater than, or equal to 0.2 OD units, was positive, while absorbance read less than 0.2 OD units was negative. A positive OD reading indicates that the patient may be infected by *Toxocara*.

Eosinophil count

Eosinophil count was performed by a laboratory technician at University Malaya Medical Center, Kuala Lumpur.

Statistical analysis

Statistical analysis in this study was performed using SPSS version 19. Chi-square test was conducted to conform the link between Anti Toxocara IgG and other variables. The level of significance selected was $p < 0,05$.

RESULTS

The (mean+SD) of age for patients was (64.19+15.107). Table 1 shows the distribution of study variables among study patients and association of these variables with *Toxocara* seroprevalence. Based on this study, there was a statistically significant association between *Toxocara* seroprevalence (*Anti Toxocara* IgG seropositivity) and pet ownership as well as having wheezing symptom in the past 12 months ($p < 0.05$). There was no statistical association between *Toxocara* seroprevalence and other study variables and outcomes among the patients under study. Besides, there was no association between *Toxocara* seroprevalence and increasing level of total IgE or eosinophilia ($p > 0.05$).

DISCUSSION

The possible role of *T. canis* in asthma is not clear. Respiratory changes occur in VLM and CT because of larval migration. According to Buijs *et al*. (1994), the anti-*Toxocara* antibodies in asthmatic children were higher than those in healthy children. Moreover, the seropositivity of *Toxocara* was higher among children aged between 11 and 15, compared to children aged between 2

Table 1. Frequency of the studied variables and their association with anti-*Toxocara* IgG seropositivity in 86 adult asthmatic patients

Variables	N (Frequency of Patients)	%	Anti-*Toxocara* IgG seropositivity n= 59 (68.6%)			
			n (%)	Exp (B) or Odds Ratios	95% C.I. for Exp (B)	Asymptomatic Significance (*p* value)
Gender				1.063	(0.378–2.991)	0.908
Female	63	73.26	68.3			
Male	23	26.74	69.6			
Race				1.099	(0.653–1.850)	0.846
Malay	24	27.91	66.7			
Chinese	23	26.74	65.2			
Indian	37	43.02	73			
Others	2	2.33	50			
Habitat				3.5	(0.409–29.973)	227
Urban	78	90.7	66.7			
Rural	8	9.3	87.5			
Education				0.904	(0.668–1.223)	0.837
None	6	6.98	66.7			
Primary school	19	22.09	68.4			
Secondary school	35	40.7	71.4			
College	9	10.47	77.8			
Diploma	1	1.16	100			
University	16	18.6	56.3			
Annual income				0.659	(0.477–0.911)	115
0–5000	50	58.14	78			
5000–10000	8	9.3	62.5			
10000–30000	11	12.79	72.7			
30000–50000	11	12.79	45.5			
50000–100000	4	4.65	25			
>100000	2	2.33	50			
Pet ownership				3.359	(1.297–8.701)	0.011
No	55	63.95	78.2			
Yes	31	36.05	51.6			
Hand washing						
No	0	0	0			
Yes	86	100	68.6			

Table 1 continued...

Variables	N (Frequency of Patients)	%	Anti-*Toxocara* IgG seropositivity n= 59 (68.6%)			
			n (%)	Exp (B) or Odds Ratios	95% C.I. for Exp (B)	Asymptomatic Significance (*p* value)
Uncooked meat				0.89	(0.279–2.838)	0.844
No	69	80.23	68.1			
Yes	17	19.77	70.6			
Ever had asthma				7.52	0	0.496
No	1	1.16	100			
Yes	85	98.84	68.2			
Smoking history				1.454	(0.313–6.741)	0.361
No	78	90.7	70.5			
Yes	3	3.49	33.3			
Ex	5	5.81	60			
Alcohol consumption				0.415	(0.46–3.739)	0.42
No	80	93.02	67.5			
Yes	6	6.98	83.3			
Have you had wheezing in the past 12 months?				4.103	(1.101–15.292)	0.027
No	23	26.74	87			
Yes	63	73.26	61.9			
In the past 4 weeks, have you had the following?	48	57.83	62.5	1.28	(0.715–2.291)	0.175
Partly controlled	16	19.28	87.5			
Uncontrolled	19	22.89	68.4			
Daytime asthma symptoms more than twice/week?				0.633	(0.230–1.743)	0.375
No	58	67.44	65.5			
Yes	28	32.56	75			
Any night waking due to asthma?				0.611	(0.198–1.887)	0.389
No	65	75.58	66.2			
Yes	21	24.42	76.2			
Reliever need for symptoms more than twice/week?				0.614	(0.231–1.629)	0.325
No	54	62.79	64.8			
Yes	32	37.21	75			
Any activity limitation due to asthma?				1.139	(0.430–3.014)	0.793
No	59	68.6	69.5			
Yes	27	31.4	66.7			

Table 1 continued...

Variables	N (Frequency of Patients)	%	Anti-*Toxocara* IgG seropositivity n= 59 (68.6%)			
			n (%)	Exp (B) or Odds Ratios	95% C.I. for Exp (B)	Asymptomatic Significance (*p* value)
PEFR or FEV1 <80% predicted or personal best				2.231	(0.134–37.058)	0.566
No	84	97.67	69			
Yes	2	2.33	50			
Have you needed any unscheduled care for your asthma, including calling in, an office visit, or an emergency department visit?				0.651	(0.225–1.884)	0.427
No	62	72.09	66.1			
Yes	24	27.91	75			
Have you been able to participate in school/work and recreational activities as desired?				0.656	(0.207–2.079)	0.472
No	15	17.86	60			
Yes	69	82.14	69.6			
If you are measuring your peak flow, has it been lower than your personal best?				0.928	(0.258–3.331)	0.908
No	71	84.52	67.6			
Yes	13	15.48	69.2			
Have you taken oral glucocorticoids ("steroids") for your asthma in the past year?				0.49	(0.126–1.902)	0.295
No	71	82.56	66.2			
Yes	15	17.44	80			
Have you been hospitalized for your asthma? If yes, how many times have you been hospitalized in the past year?				0.415	(0.46–3.739)	0.42
No	80	93.02	67.5			
Yes	6	6.98	83.3			
Have you been admitted to the intensive care unit or been intubated because of your asthma?				0.313	(0.65–1.512)	0.132
No	72	83.27	65.3			
Yes	14	16.28	85.7			
If yes, did this occur within the past five years?				0.51	(0.101–2.581)	0.409
No	76	88.37	67.1			
Yes	10	11.63	80			
Short acting bronchodilators (PRN)				8.388	0	113
No	5	5.95	100			
Yes	79	94.05	65.8			

Table 1 continued...

Variables	N (Frequency of Patients)	%	Anti-*Toxocara* IgG seropositivity n= 59 (68.6%)			
			n (%)	Exp (B) or Odds Ratios	95% C.I. for Exp (B)	Asymptomatic Significance (p value)
Low dose ICS				0.667	(0.165–2.691)	0.567
No	72	85.71	66.7			
Yes	12	14.29	75			
Long acting beta 2 agonist and low dose ICS				1.4	(0.518–3.782)	0.506
No	60	71.43	70			
Yes	24	28.57	62.5			
Low acting beta 2 agonist and high dose ICS				0.841	(0.336–2.108)	0.712
No	38	45.24	65.8			
Yes	46	54.76	69.6			
Long acting muscarinic antagonist (e.g. titropium)				0.766	(0.186–3.148)	0.711
No	73	86.9	67.1			
Yes	11	13.1	72.7			
Leukotriene receptor antagonist (montelukast)				0.432	(0.170–1.100)	0.076
No	38	45.24	57.9			
Yes	46	54.76	76.1			
Theophyline (e.g. Neulin SR)				0.667	(0.165–2.691)	0.567
No	72	85.71	66.7			
Yes	12	14.29	75			
Oral prednisolone (if yes, please specify dose per day)				0	0	0.325
No	82	97.62	67.1			
Yes	2	2.36	100			
Immunoglobulin E monocloonal antibody (e.g. omalizumb)				0	0	0.497
No	82	98.8	68.3			
Yes	1	1.2	100			
IgE				0.448	(0.144–1.399)	0.161
Allergy free adult < 100 IU/ml	15	17.44	53.3			
Allergy positive adult > 100 IU/ml	71	82.56	71.8			
Eosinophil count (0.02–0.50)				0.865	(0.309–2.419)	0.782
Negative	62	72.09	67.7			
Positive	24	27.91	70.8			

(Chi-square, $p < 0{,}05$).

and 10. Fernando *et al*. (2009) indicated that 29% of children with bronchial asthma showed *Toxocara* seropositive manifestation. High eosinophilia was detected in 86% of these asthmatic children (Fernando *et al*., 2009). According to Mendonça *et al*. (2012), the prevalence of *Toxocara* in children from Salvador area in Brazil was found to be 47%. There are few studies and reports indicating the relationship between asthma and toxocariasis among adults. Feldman *et al*. (1992) reported a high level of anti-*Toxocara* IgG in a 48-year-old asthmatic patient with a hypereosinophilic syndrome. According to Buijs *et al*. (1995), there was a marginal relation between eczema and *Toxocara* seropositivity. Ehrhard *et al*. (1979) observed transient rash, urticaria and hypodermic nodules in 23% of seropositive children and 29% of seropositive adults.

The rate of toxocariasis across Malaysia has been indicated to be around 20%. This study focused on adult asthmatic patients, differ to previous studies conducted in Malaysia (Hakim *et al*., 1997) and in Brazil (Mendonça *et al*., 2012) while they studied the relationship between asthma and toxocariasis in children. Since biopsy, as the gold standard diagnostical test of toxocariasis, is laborious and time-consuming; thus, serological diagnosis is a more reliable test for detecting *Toxocara*. In the current study, 86 serum samples of adult asthmatic patients were obtained from UMMC hospital. *Toxocara* IgG ELISA was done to confirm toxocariasis. Out of 86 patients, 59 (68.60%) were positive with toxocariasis, and 27 (31.39%) were negative. We found no association between *Toxocara* infection and major immunological reactions such as marked eosinophila and IgE response. In this study, the risk factors that include age, gender, habitat, education level, pet ownership, washing hands before meals, raw meat consumption, family history of asthma disease and other variables, have been investigated based on previously published papers as well as ISSAC questionnaire. Similar to Sharghi *et al*. (2001), our results did not indicate any links between asthma and toxocariasis. We found that 36.05% of asthmatic patients had pets. This result is lower in percentage when compared to the study carried out by Mendonça *et al*. (2013), where 66.6% of the contact with puppies and 60% of the contact with adult dogs were reported positive. Based on the statistical analyses conducted by SPSS version 19, we found a significant relatiomship between Toxocara seroprevalence and pet ownership, as well as the pathogenesis of wheezing in the past 12 months. No relations were detected between Toxocara seroprevalence and other variables among adult asthmatic patients. One of the limitation of our study was lack of control in our research. Besides, *Toxocara*-specific IgE level were not tested in our study since we had only Total-IgE test.

CONCLUSION

In conclusion, this study confirmed detection of positive *anti Toxocara* IgG in 68.6% of adult asthmatics. Besides, the present study demonstrated a statistically significant relationship between Toxocara seropositivity and pet ownership, as well as the pathogenesis of wheezing in asthmatic adults' during the last 12 months. Toxocara seroprevalence has not been found associated with other variables among patients. No signifaicant correlation has been found between positive *anti Toxocara* IgG signs and high level of total IgE and eosinophilia concentration.

Conflict of interest

The authors declarethat they have no conflict of interest.

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