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Parasitic Infection as a Risk Factor for Childhood Asthma in Upper Egypt

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Abstract- *Background:* Asthma and allergic diseases are serious public health problems in many middle and low-income countries. We examined the relationship between parasitic infection and the development and severity of asthma among children living in Assiut Governorate, Upper Egypt.

Methods: A cross sectional study was conducted on 140 children suffering from bronchial asthma (78 males and 62 females) aged from 5 to 14 years attending Assiut University Children Hospital. As well as 70 apparently healthy children with matched age and sexas controls. Beside meticulous history taking and clinical examination all patients and controls undergone; pulmonary function test, stool analysis, antibodies to Toxocara canis, antibodies to Ascaris lumbricoides,IL-5 level and Leukotriene E4.

Results: Ascaris lumbricoides and Toxocara canis infections were detected in sera of 26 (18.6%), 26 (18.6%) patients respectively, whereas Giardia infection was detected in stool of 28 (20%) of patients. Among patients infected with Ascaris 15,9, and 2 patients had severe, moderate and mild asthma respectively.

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Results: Ascaris lumbricoides and Toxocara canis infections were detected in sera of 26 (18.6%), 26 (18.6%) patients respectively, whereas Giardia infection was detected in stool of 28 (20%) of patients. Among patients infected with Ascaris 15,9, and 2 patients had severe, moderate and mild asthma respectively. While among patients infected with Toxocara 13,10, and 3 patients had severe, moderate and mild asthma respecively., As regard patients infected with Giardia 15,12 and1 patients had severe, moderate and mild asthma respectively. Among controls Giardia infection was detected in stool of 4 children (2.8%). Among controls Giardia infection was detected in stool of 4 children (2.8%).

Conclusion: Infection with Ascaris, Toxocara and Giardia is more common among asthmatic children so infection with these parasites may be a risk factor for bronchial asthma among Upper Egyptian children.

Briefpoints

What is known: The multidimensional relationship between parasitic infections and asthma. and atopy.

The immunomodulatory effects of some parasites and their protective effects upon asthma.

A.lumbricoides eggs were associated with an increased prevalence of asthma.

What is to add: Infection with *Ascaris*, *Toxocara* and *Giardia* is more common among asthmatic children than healthy children.

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Infection with these parasites may be a risk factor for development of bronchial asthma among Upper Egyptian children.

Infection with these parasites may be a risk factor for increased asthma severity among these asthmatic children.

I. Background

Asthma as one of the most common allergic diseases causes major public health problem in many developed and developing countries. Asthma is characterized by chronic inflammation of the airways and it is one of the most common diseases among children worldwide. Asthma affects 300 million people worldwide[1].

What is known The multidimensional relationship between parasitic infections and asthma. and atopy has been previously reported in many studies[2]. However, the association between parasitic infection and childhood asthma and atopy remains controversial[3].

The immunomodulatory effects of some parasites and their protective effects upon asthma had been addressed in many studies. On the other hand *A. lumbricoides* eggs were associated with an increased prevalence of asthma and anti-Ascaris IgE had been reported to be associated with an increased risk of asthma symptoms[4].

Human toxocariasis is a cosmopolite helminthic zoonosis caused by *Toxocara canis* and *Toxocara cati*, which are common roundworms of dogs and cats, respectively[5]. It has been reported that an increased risk of wheeze in some populations may be associated *Toxocara* infections and that may be caused by the host response to the parasite or by parasite-enhanced Th2 responses to aeroallerges[6].

Activation of Th2-type immune response which takes place in giardiasis and proved by enhanced IgE production pointed to and confirmed its association with allergy. Also IgE production is larger and more severe in allergy-complicated giardiasis than that of uncomplicated cases[7].

The aim of this study was to assess the relationship between certain parasitic infection and the development and severity of asthma among children living in Assiut Governorate, Upper Egypt.

What is to add Infection with *Ascaris*, *Toxocara* and *Giardia* is more common among asthmatic children so

infection with these parasites may be a risk factor for bronchial asthma among Upper Egyptian children.

II. Materials and Methods

A cross-sectional descriptive study was performed which included 140 children with persistent bronchial asthma (78 males and 62 females) recruited at Assiut University Children Hospital, during the period from January, 2015 to January, 2016. Their ages were ranging from 5 to 14 years. As well as 70 apparently healthy children with matched age and sex were participated as controls.

Inclusion criteria

Agreement to participate; recurrent episodes of coughing, wheezing and breathlessness, especially if aggravated or triggered by exposure to inhaled allergens, viral infection or exercise and relieved by the use of bronchodilators, corticosteroids or subcutaneous epinephrine. Children should not take anti-parasitic medication in the previous 6 months and provided three samples for parasite tests on alternate days.

Exclusion criteria

Not meeting all inclusion criteria, other causes of wheezy chest such as: tuberculosis, foreign body inhalation, bronchiectasis, bronchopneumonia or any other anatomic or congenital malformations

All cases and controls included in the study were subjected to:

i. Meticulous history taking including
ii. Thorough clinical examination
iii. *Laboratory investigations:* pulmonary function tests (PEFR and FEV_1), stool examination, absolute eosinophilic count, IgE antibodies to *Ascaris lumbricoides by serology*, IgG antibodies to *Toxocara canis by ELISA*, serum IL-5 level and urinary Leukotriene E4 in urine.

As regard the severity of asthma, we classified patients into 3 groups according to the Global Initiative for Asthma 2002[8].

Group I: 20 patients had mild persistent asthma (12 males and 8 females).

Group II: 60 patients had moderate persistent asthma (34 males and 28 females).

Group III: 60 patients had severe persistent asthma (32 males and 26 females).

a) Stool Examination

We collected stool samples from all participants in sterile clean stool plastic disposable cups with lids labeled with the patient's serial number, name, age, and sex, group of BA and date of collection. Within half an hour all collected samples were examined parasitologically. We used iodine and lactophenol cotton blue for direct wet smear. Then, fomol-ether sedimentation was done to the stool samples and examined.

b) Urinary Leukotriene E4

Urinary LTE4 levels were assessed using the commercially available enzyme immunoassay (Cayman Chemical; AnnArbor, MI, USA).

c) Blood Samples

We collected blood samples from the participants by venipuncture. Cellular assay (AEC) was performed (Eosinophilia corresponded to levels above 400/mm3), then the serum samples collected were stored at -70°C until the serological analysis.

d) Total IgE levels

We used ELISA to measure total IgE levels where levels above 200 IU/mL were considered high. All samples were measured in duplicate.

e) Human IL-5 Level Assay

Human enzyme-linked immunosorbent assay kitare used to measureIL-5 levels (Biosource International, Inc., Camarillo, California, USA), according to the manufacturer's instructions. The lowest level of detection of IL-5 was 2 pg/mL. The intra-assay coefficient of variation was 7.4%, and the inter-assay coefficient of variation was 10%.

f) Detection of Ascaris lumbricoides Infection in seology

We measured specific IgE levels against Ascaris by the CAP-FEIA fluoro enzyme immunoassay method (Phadia AB, Uppsala, Sweden).

g) Detection of Toxocara canis Infection in serology

Were prepared excretory/secretory antigens from laboratory cultivated second stage larvae of *T. canis* according to the method of Sugan et al.[9].The antigen was stored at -70°C until used as a crude antigen. We used ELISA technique to detect IgG against *T. canis* according to Van Kanpen[10]. ELISA plates (Flow Lab. Cat. No., 76-321-05) were coated by the prepared antigen.

h) Statistical analysis

We used SPSS statistics version 22 (IBM Corporation, NY, USA) to analyze our data. Values were expressed as means and standard deviation (SD). Qualitative variables were presented as number (n) and percentage (%). We used Chi-square test to compare qualitative variables between groups. Unpaired *t*-test and Mann-Whitney "U" tests were used to compare quantitative variables. Anti-*Ascaris* IgE was classified into quartiles based on the distribution of the study participants.

III. Results

Regarding pulmonary functions, all groups of patients showed significantly lower PEFR% and FEV_1%

than controls but only FEV_1% was insignificantly lower in mild group than controls. Regarding AEC, all patients showed significantly higher values than controls. IL-5 was significantly higher in different groups of patients than controls. Furthermore, asthmatic patients whatever collectively or subgroups showed significantly higher urinary LTE_4 levels than controls (Table 1).

Table (1): Pulmonary functions, A.E.C., serum IL-5 and urinary LTE4 of studied patients versus controls

	I Patients (n: 140)	II Mild patients (n:20)	III Moderate patients (n:60)	IV Severe patients (n: 60)	V Controls (n: 70)	P.value I vs V	P.value II vs V	P.value III vs V	P.value IV vs V
1- Pulmonary functions: - PEFR (%) (mean ± SD)	57.540 ± 15.058	71.800± 14.551	61.933± 9.958	48.400 ± 14.075	98.350 ± 0.587	0.000 HS	0.000 HS	0.000 HS	0.000 HS
- FEV_1 (%) (mean ± SD)	62.140 ± 15.554	85.900 ± 6.350	67.967 ± 5.236	48.400 ± 10.516	94.300 ±19.850	0.000 HS	0.206 NS	0.000 HS	0.000 HS
3- A.E.C (mean ± SD)	731.930 ± 244.377	332.600 ± 100.603	643.467 ± 90.239	953.500 ± 122.081	121.950 ± 51.635	0.000 HS	0.000 HS	0.000 HS	0.000 HS
4- IL-5 (pg/ml) (mean ± SD)	46.3 ±31.7	13.300 ± 3.683	26.850 ± 4.957	74.333 ± 30.335	6.725 ± 3.952	0.000 HS	0.000 HS	0.000 HS	0.000 HS
5- LTE_4 (pg/ml) (mean ± SD)	394.9 ±287.2	110.125 ±49.441	269.038 ± 47.010	656.333 ± 259.756	35.222 ± 5.044	0.001 MS	0.000 HS	0.000 HS	0.000 HS

PEFR: Peak Expiratory Flow Rate
FEV1: Forced Expiratory Volume in 1 second
IL-5: Interleukin-5
LTE4: Leukotriene E4
A.E.C: Absolute Eosinophilic Count
ABG: Arterial Blood Gases
S: Significant (P<0.05)
MS: Moderately significant (P<0.005)
HS: Highly significant P<0..001)
NS: Non sngificant (P>0.05)

Table (2) Patients with severe and moderate asthma showed significantly lower PEFR% and FEV_1% than mild patients and also severe patients showed significantly lower PEFR% and FEV_1 % than moderate patients.

As regard AEC, serum IL-5 and urinary LTE_4, severe and moderate asthmatics showed significantly higher values than mild patients. Also, severe patients showed significantly higher values compared to moderate patients.

Table (2): Pulmonary functions, A.E.C., serum IL-5 and urinary LTE4 of asthmatic children in relation to severity

	I Mild patients (n 20)	II Moderate patients (n: 60)	III Severe patients (n:60)	P. value I vs II	P. value I vs III	P. value II vs III
1- Pulmonary functions: - PEFR (%) (mean ± SD)	71.800 ± 14.551	61.933 ± 9.958	48.400 ± 14.075	0.021 S	0.000 HS	0.000 HS
- FEV_1 (%) (mean ± SD)	85.900 ± 6.350	67.967 ± 5.236	48.400 ± 10.516	0.000 HS	0.000 HS	0.000 HS
3- A.E.C (mean ± SD)	332.600 ± 100.603	643.467 ± 90.239	953.500 ± 122.081	0.000 HS	0.000 HS	0.000 HS
4- IL-5 (pg/ml) (mean ± SD)	13.300 ± 3.683	26.850 ± 4.957	74.333 ± 30.335	0.000 HS	0.000 HS	0.000 HS
5- LTE_4 (pg/ml) (mean ± SD)	110.125 ± 49.441	269.038 ± 47.010	656.333 ± 259.756	0.000 HS	0.000 HS	0.000 HS

PEFR: Peak Expiratory Flow Rate
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A.E.C: Absolute Eosinophilic Count
ABG: Arterial Blood Gases
S: Significant (P<0.05)
MS: Moderately significant (P<0.005)
HS: Highly significant (P<0..001)
NS: Non sngificant (P>0.05)

Table (3) Among the studied patients *Ascaris lumbricoides* and *Toxocara* infections showed similar occurrence where they were detected in sera of 26 (18.6%), whereas *Giardia* infection was detected in stools of 28(20%) of patients. Among 26 patients infected with *Ascaris* 15 patients have severe asthma, 9 patients have moderate asthma and 2 patients have mild asthma while among 26 patients infected with

Toxocara 13 patients have severe asthma, 10 patients have moderate asthma and 3 patients have mild asthma. As regard 28 patients infected with *Giardia* 15 patients have severe asthma, 12 patients have moderate asthma and 1 patient have mild asthma. Among controls only *Giardia* infection was detected in stools of 4 (2.8%) of controls. Polyparasitism was not detected among patients or controls.

Table (3): Prevalence of parasitic infection among the examined asthmatic patients and controls

Parasite	Patients (n)	(n:140) (%)	Controls (n)	(70) (%)
Ascaris lumbricoides	(26): 15 severe, 9 moderate, 2 mild	18.6	0	0
Toxocara canis	(26) 13 severe 10 moderate 3 mild	18.6	0	0
Giardia lamblia	28 15 severe 12 moderate 1 mild	20	4	2.8
Polyparasitism	0	0	0	0

Table (4) Regarding pulmonary functions, no significant difference was found between patients who were positive and those who were negative regarding *Ascaris* infection whereas, both groups showed significantly lower values of PEFR% and FEV_1 % compared to controls.

Regarding AEC, patients with positive *Ascaris* infection showed significantly higher value than those with negative *Ascaris* infection. Both groups showed significantly higher values of AEC compared to controls. Regarding serum IL-5 and urinary LTE_4, patients who were positive for *Ascaris* infection showed significantly higher values than those with negative *Ascaris* infection. Furthermore, both groups showed significantly higher values of serum IL-5 and urinary LTE_4 compared to controls.

Table (4): Pulmonary functions, A.E.C, serum IL-5 and urinary LTE4 in patients with +ve and –ve *Ascaris lumbricoides* infection versus controls.

	I Patients with +ve Ascaris infection by serology (n : 26)	II patients with –ve Ascaris infection by serology (n:114)	III Controls (n : 70)	P. value		
				I vs III	II vs III	I vs II
1- Pulmonary functions: - PEFR (%) (mean ± SD)	45.385 ± 11.057	58.260 ± 15.822	98.350 ± 0.587	0.000 HS	0.000 HS	0.406 NS
- FEV_1 (%) (mean ± SD)	54.846 ± 12.096	63.810 ± 15.860	94.300 ± 19.850	0.000 HS	0.000 HS	0.060 NS
3- A.E.C (mean ± SD)	888.000 ± 249.733	696.330 ± 230.814	121.950 ± 51.635	0.000 HS	0.000 HS	0.010 S
4- IL-5 (pg/ml) (mean ± SD)	62.769 ± 37.468	41.272 ± 30.332	6.725 ± 3.951	0.000 HS	0.000 HS	0.031 S
5- LTE_4 (pg/ml) (mean ± SD)	665.833 ± 308.584	340.950 ± 253.548	35.222 ± 5.044	0.000 HS	0.001 MS	0.009 MS

PEFR: Peak Expiratory Flow Rate
FEV1: Forced Expiratory Volume in 1 second
IL-5: Interleukin-5
LTE4: Leukotriene E4
A.E.C: Absolute Eosinophilic Count
ABG: Arterial Blood Gases
S: Significant (P<0.05)
HS: Highly significant (P<0..001)
NS: Non sngificant (P>0.05)
MS: Moderately significant (P<0.005)

Table (5) Regarding pulmonary function, no significant difference was found between patients with positive and negative *Toxocara* infection whereas, both groups showed significantly lower values of PEFR% and FEV_1 % compared to controls.

Regarding AEC, patients who were positive for *Toxocara* infection showed significantly higher value than those with negative *Toxocara* infection. Both groups showed significantly higher values of AEC compared to controls. Regarding serum IL-5 and urinary LTE_4, patients who were positive for *Toxocara* infection showed significantly higher values than those with negative *Toxocara* infection. Furthermore, both groups showed significantly higher values of serum IL-5 and urinary LTE_4 compared to controls.

Table (5): Pulmonary functions, A.E.C, serum IL-5 and urinary LTE4 in patients with +ve and –ve *Toxocara canis* infection versus controls

	I Patients with +ve Toxocare infection by serology (n :26)	II patients with –ve Toxocara infection by serology (n:114)	III Controls (n : 70)	P. value		
				I vs III	II vs III	I vs II
1- Pulmonary functions: - PEFR (%) (mean ± SD)	45.385 ± 11.057	58.260 ± 15.822	98.350 ± 0.587	0.000 HS	0.000 HS	0.406 NS
- FEV_1 (%) (mean ± SD)	54.846 ± 12.096	63.810 ± 15.860	94.300 ± 19.850	0.000 HS	0.000 HS	0.060 NS
3- A.E.C (mean ± SD)	888.000 ± 249.733	696.330 ± 230.814	121.950 ± 51.635	0.000 HS	0.000 HS	0.010 S
4- IL-5 (pg/ml) (mean ± SD)	62.769 ± 37.468	41.272 ± 30.332	6.725 ± 3.951	0.000 HS	0.000 HS	0.031 S
5- LTE_4 (pg/ml) (mean ± SD)	665.833 ± 308.584	340.950 ± 253.548	35.222 ± 5.044	0.000 HS	0.001 MS	0.009 MS

PEFR: Peak Expiratory Flow Rate
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ABG: Arterial Blood Gases
S: Significant (P<0.05)
HS: Highly significant (P<0..001)
NS: Non sngificant (P>0.05)
MS: Moderately significant (P<0.005)

Table (6) Regarding pulmonary functions, *Giardia* positive patients showed significantly lower PEFR % and FEV_1% than patients with negative *Giardia* infection. Furthermore, both groups showed significantly lower PEFR% and FEV_1% compared to controls

Regarding AEC and urinary LTE_4, patients with positive *Giardia* infection showed significantly higher values than patients with negative *Giardia* infection. Furthermore, both groups showed significantly higher values than controls. Regarding serum IL-5, patients with negative *Giardia* infection showed significantly higher value than patients with positive *Giardia* infections. Both groups showed significantly higher value than controls.

Table (6): Pulmonary functions, A.E.C, serum IL-5 and urinary LTE4 in patients with +ve and –ve *Giardia* infection versus controls

	I Patients with +ve Giardial infection (n : 28)	II Patients with –ve Giardial infection (n:112)	III Controls (n : 70)	P. value		
				I vs III	II vs III	I vs II
1- Pulmonary functions: - PEFR (%) (mean ± SD)	53.930 ± 16.657	58.450 ± 14.653	98.350 ± 0.587	0.000 HS	0.000 HS	0.025 S
- FEV_1 (%) (mean ± SD)	68.210 ± 13.174	64.750 ± 15.088	94.300 ± 19.850	0.000 HS	0.000 HS	0.004 MS
3- A.E.C (mean ± SD)	970.360 ± 171.471	672.320 ± 223.347	121.950 ± 51.635	0.000 HS	0.000 HS	0.000 HS
4- IL-5 (pg/ml) (mean ± SD)	66.500 ± 40.553	93.955 ± 28.281	6.725 ± 3.952	0.000 HS	0.000 HS	0.006 MS
5- LTE_4 (pg/ml) (mean ± SD)	708.750 ± 352.489	305.482 ± 191.151	35.222 ± 5.044	0.000 HS	0.000 HS	0.000 HS

PEFR: Peak Expiratory Flow Rate
FEV1: Forced Expiratory Volume 1 second
IL-5: Interleukin-5
LTE4: Leukotriene E4
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S: Significant (P<0.05)
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NS: Non sngificant (P>0.05)

IV. Discussion

Asthma is a chronic lung disease characterized by reversible airway obstruction, inflammation, and bronchial hyperresponsiveness[11)]

In this study, the relationship between *Ascaris lumbricoides*, *Toxocara canis*, *Giardia lamblia infections* and development and severity of childhood asthma has been studied As regard the association of parasitic infections and bronchial asthma, ascariasis were detected in the sera of 26 patients (18.6%) and toxocariasis showed similar occurrence, whereas giardiasis was detected in the stools of 28 patients (20%). On the other hand only giardiasis was detected in stools of 4 (2.8%) of controls. It is possible for these parasites to be important risk factors in our communities. Our study revealed that parasitic infections with *Ascaris*, *Toxocara* and *Giardia* were more common among severely asthmatic children than among moderately and mildly asthmatics. This was supported by the finding of significantly higher levels of AEC, urinary LTE4 and IL-5 in *Ascaris*, *Toxocara* and *Giardia* positive asthmatics than negative ones. Also, pulmonary functions were insignificantly lower in the earlier than the latter (Table 4, 5, 6).

These results were in line with previous studies who reported the increased prevalence of parasitic infections and possible influence of parasitic infections on the development and severity of allergic conditions in the tropical environment [12,13)].

Our results were in agreement with systematic review and met analysis of 30 cross-sectional studies found that *A. lumbricoids* infection appeared to increase asthma risk[14)].

Previous studies have provided conflicting evidence regarding relationship between parasitic infections and development of asthma. These studies showed that helminth infection can inhibit[15)], cause[16)] or is unrelated to asthma[17)]. The role of anti-*Ascaris* IgE in the development of asthma is not clear. One possible explanation for the relationship is that elevated anti-*Ascaris* IgE levels are associated with larval migration after re-infection, as *Ascaris* migrates through the lungs during maturation and causes pulmonary infiltrates of Th2 immunity and episodic airway obstruction associated with wheezing[18)]. Repeated *Ascaris* infections and larval migration due to high rate of infection could increase the risk of asthma symptoms. Another explanation is that anti-*Ascaris* IgE acts as IgE specific to common inhaled aero-antigens directly triggering mast cell activation[19)]. This finding was supported by two other studies[20,21)]. The third explanation is that the higher anti-*Ascaris* IgE levels in the wheezing group simply mean that atopic children produce more anti-*Ascaris* IgE in response to *Ascaris*infection[22)]. Parallel to this observation, Heukelbach et al. reported that exposure to *Toxocara* infection was suggested to be a possible risk factor for asthma[23)]. One good explanation for that is, *Toxocara* species can cause allergy (asthma) in man by liberation of larval excretory/secretory antigens. Moreover, *Toxocara* was found to induce polyclonal activation of IgE producing B-cells as well as peripheral and tissue eosinophilia[24)]. these phenomena are commonly occured with IgE mediated diseases such as allergy.

There is hypothesis that many zoonotic helminth infections cannot develop to maturity in the human host and therefore, larvae may migrate for prolonged periods in the tissues. Examples are infections with *Toxocara* spp, *Ascaris suum*, and dog hookworms. Such infections cause allergic type syndromes such as cutaneous and visceral larva migrans[25)]. Damage of these tissues can be caused by allergic inflammation directed against the migrating larvae associated with failure of immune regulation during such infections probably because host and parasite have not co-evolved.

Our results were in line with Di Prisco et al.[26)] who found that *Giardia lamblia* parasitized children showed significantly higher levels of both total and specific serum IgE antibodies against allergens compared both with the non-parasitized group and those infected with parasites other than *Giardia*. The investigators concluded that there was a clear relation between giardiasis and allergy, possibly because infection by this protozoan enhanced sensitization towards food antigens, due to increased antigen penetration through damaged intestinal mucosa.

It has been reported that activation of the immune system takes occurs in giardiasis. It is wider and more severe in allergy-complicated giardiasis than that of uncomplicated cases, most probably due to non-invasive character of *G. lamblia*. Enhanced IgE production pointed to Th2-type immune response and confirms its association with allergy[6)].

V. Conclusion

Ascaris, *Toxocara* and *Giardia infections* are more common among asthmatic children compared to healthy children and they were significantly associated with the disease severity therefore, infection with these parasites may be a risk factor for the development and severity of bronchial asthma among children in Upper Egypt.

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