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

By Alameldin M. Abdallah MD, Randa E.Abd-Elkader MD & Doaa A.Yones MD

Assiut University

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

Alameldin M. Abdallah MD a, Randa E.Abd-Elkader MD b & Doaa A.Yones MD c

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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 respectively. 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 has been previously reported in many studies. However, the association between parasitic infection and childhood asthma and atopy remains controversial.

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.

Human toxocariosis is a cosmopolite helminthic zoonosis caused by Toxocara canis and Toxocara cati, which are common roundworms of dogs and cats, respectively. 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 aeroallergens.

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.

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 FEV₁), 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, formol-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; Ann Arbor, 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 -70ºC 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 serology

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

We 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 -70ºC 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 FEV₁%
than controls but only FEV₁% 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 LTE₄ levels than controls (Table 1).

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

<table>
<thead>
<tr>
<th>Patients (n: 140)</th>
<th>I Mild patients (n:20)</th>
<th>II Moderate patients (n:60)</th>
<th>III Severe patients (n: 60)</th>
<th>IV Controls (n: 70)</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I- Pulmonary functions: PEFR (%) (mean ± SD)</td>
<td>57.540 ± 15.058</td>
<td>71.800 ± 14.551</td>
<td>61.933 ± 9.958</td>
<td>48.400 ± 14.075</td>
<td>48.350 ± 0.587</td>
</tr>
<tr>
<td>- FEV₁ (%) (mean ± SD)</td>
<td>62.140 ± 15.554</td>
<td>85.900 ± 6.350</td>
<td>67.967 ± 5.236</td>
<td>48.400 ± 10.516</td>
<td>94.300 ± 19.850</td>
</tr>
<tr>
<td>III- A.E.C (mean ± SD)</td>
<td>731.930 ± 244.377</td>
<td>332.600 ± 100.603</td>
<td>643.467 ± 90.239</td>
<td>953.500 ± 122.081</td>
<td>121.950 ± 51.635</td>
</tr>
<tr>
<td>IV- IL-5 (pg/ml) (mean ± SD)</td>
<td>46.3 ± 31.17</td>
<td>13.300 ± 3.638</td>
<td>26.850 ± 4.957</td>
<td>74.333 ± 30.335</td>
<td>6.725 ± 3.952</td>
</tr>
<tr>
<td>V- LTE₄ (pg/ml) (mean ± SD)</td>
<td>394.9 ± 287.2</td>
<td>110.125 ± 49.441</td>
<td>269.038 ± 47.010</td>
<td>656.333 ± 259.756</td>
<td>35.222 ± 5.044</td>
</tr>
</tbody>
</table>

PEFR: Peak Expiratory Flow Rate
FEV₁: Forced Expiratory Volume in 1 second
A.E.C: Absolute Eosinophilic Count
IL-5: Interleukin-5
LTE₄: Leukotriene E₄
ABG: Arterial Blood Gases
S: Significant (P<0.05)
MS: Moderately significant (P<0.005)
HS: Highly significant (P<0.001)
NS: Non significant (P>0.05)

Table (2) Patients with severe and moderate asthma showed significantly lower PEFR% and FEV₁% than mild patients and also severe patients showed significantly lower PEFR% and FEV₁% than moderate patients.

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

<table>
<thead>
<tr>
<th>Patients (n: 20)</th>
<th>I Mild patients (n:20)</th>
<th>II Moderate patients (n:60)</th>
<th>III Severe patients (n: 60)</th>
<th>P. value</th>
</tr>
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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

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Patients (n)</th>
<th>Controls (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascaris lumbricoides</td>
<td>(26): 15 severe, 9 moderate, 2 mild</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td>Toxocara canis</td>
<td>(26): 13 severe, 10 moderate, 3 mild</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>28: 15 severe, 12 moderate, 1 mild</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyparasitism</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>I Patients with +ve Ascaris infection by serology (n: 26)</th>
<th>II Patients with –ve Ascaris infection by serology (n:114)</th>
<th>III Controls (n: 70)</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I vs III</td>
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<td></td>
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<td>1- Pulmonary functions: PEFR (%) (mean ± SD)</td>
<td>45.385 ± 11.057</td>
<td>58.260 ± 15.822</td>
<td>98.350 ± 0.587</td>
<td>0.000 HS</td>
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<tr>
<td></td>
<td>- FEV1 (%) (mean ± SD)</td>
<td>54.846 ± 12.096</td>
<td>63.810 ± 15.860</td>
<td>94.300 ± 19.850</td>
</tr>
<tr>
<td>3- A.E.C (mean ± SD)</td>
<td>888.000 ± 249.733</td>
<td>696.330 ± 230.814</td>
<td>121.950 ± 51.635</td>
<td>0.000 HS</td>
</tr>
<tr>
<td>4- IL-5 (pg/ml) (mean ± SD)</td>
<td>62.769 ± 37.468</td>
<td>41.272 ± 30.322</td>
<td>6.725</td>
<td>0.000 HS</td>
</tr>
<tr>
<td>5- LTE4 (pg/ml) (mean ± SD)</td>
<td>665.833 ± 308.584</td>
<td>340.950 ± 253.548</td>
<td>35.222</td>
<td>0.000 HS</td>
</tr>
</tbody>
</table>

PEFR: Peak Expiratory Flow Rate  A.E.C: Absolute Eosinophilic Count  HS: Highly significant (P<0.001)
FEV1: Forced Expiratory Volume in 1 second  ABG: Arterial Blood Gases  NS: Non significant (P>0.05)
IL-5: Interleukin-5  S: Significant (P<0.05)  MS: Moderately significant (P<0.005)
LTE4: Leukotriene E4

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 FEV1 % compared to controls.
Regarding AEC, patients who were positive for *Toxocara* infection showed significantly higher values than those with negative *Toxocara* infection. Both groups showed significantly higher values of AEC compared to controls. Regarding serum IL-5 and urinary LTE4, 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 LTE4 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

<table>
<thead>
<tr>
<th>Patients with +ve <em>Toxocara</em> infection by serology (n : 26)</th>
<th>Patients with –ve <em>Toxocara</em> infection by serology (n: 114)</th>
<th>Controls (n: 70)</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Pulmonary functions:</td>
<td></td>
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</tbody>
</table>

PEFR: Peak Expiratory Flow Rate  
A.E.C: Absolute Eosinophilic Count  
ABG: Arterial Blood Gases  
IL-5: Interleukin-5  
LTE4: Leukotriene E4  

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

<table>
<thead>
<tr>
<th>Patients with +ve <em>Giardia</em> infection (n : 28)</th>
<th>Patients with –ve <em>Giardia</em> infection (n: 112)</th>
<th>Controls (n: 70)</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Pulmonary functions:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- PEFR (%) (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53.930 ± 16.657</td>
<td>58.450 ± 14.653</td>
<td>98.350 ± 0.587</td>
<td>0.000</td>
</tr>
<tr>
<td>68.210 ± 13.174</td>
<td>64.750 ± 15.088</td>
<td>94.300 ± 19.850</td>
<td>0.000</td>
</tr>
<tr>
<td>3- A.E.C (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>970.360 ± 171.471</td>
<td>672.320 ± 223.347</td>
<td>121.950 ± 51.635</td>
<td>0.000</td>
</tr>
<tr>
<td>66.500 ± 40.553</td>
<td>93.955 ± 28.281</td>
<td>6.725 ± 3.952</td>
<td>0.000</td>
</tr>
<tr>
<td>5- LTE4 (pg/ml) (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>708.750 ± 352.489</td>
<td>305.482 ± 191.151</td>
<td>35.222 ± 5.044</td>
<td>0.000</td>
</tr>
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IV. Discussion

Asthma is a chronic lung disease characterized by reversible airway obstruction, inflammation, and bronchial hyperresponsiveness.

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, ascariosis were detected in the sera of 26 patients (18.6%) and toxocariosis 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 positive controls. 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.

Our results were in agreement with systematic review and meta-analysis of 30 cross-sectional studies found that A. lumbricoides infection appeared to increase asthma risk.

Previous studies have provided conflicting evidence regarding relationship between parasitic infections and development of asthma. These studies showed that helminth infection can inhibit, cause or is unrelated to asthma. 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 reinfection, as Ascaris migrate through the lungs during maturation and causes pulmonary infiltrates of Th2 immunity and episodic airway obstruction associated with wheezing. Repeated Ascaris infections and larval migration due to high risk 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. This finding was supported by two other studies. 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. Parallel to this observation, Heukelbach et al. reported that exposure to Toxocara infection was suggested to be a possible risk factor for asthma. 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. These phenomena are commonly occurred 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. 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. 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.

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.

References

1. Braman SS. The global burden of asthma. Chest 2006; 130: 4S–12S.


