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

Doaa A.Yones MD

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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

Index terms—

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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 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. 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.

1 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.

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.

2 I.

Background sthma 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.

3 II.

4 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.

5 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.

6 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 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).

7 All cases and controls included in the study

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).

8 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.

9 b) Urinary Leukotriene E4

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

10 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^{o}C until the serological analysis.

11 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.

12 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%.

13 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).

14 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^{o}C until used as a crude antigen. We used ELISA technique to detect IgG against T. canis according to Van **??**anpen 10) . ELISA plates (Flow Lab. Cat. No., 76-321-05) were coated by the prepared antigen.

15 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.

16 III.

17 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 **??**). **??**) 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. **??**) 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 (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.

18 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 **??**, 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 Ascarisinfection 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 coevolved.

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 noninvasive character of G. lamblia. Enhanced IgE production pointed to Th2-type immune response and confirms its association with allergy 6) .

V.

19 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 [1]

[1]

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	I Patients (n: 140)	II Mild patients (n:20)	III patients (n:60)	IV Severe patients (n: 60)	V Controls (n: 70)	P.value I vs V	II vs V	III 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
-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
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
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
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

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

Figure 1: Table (1

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Figure 2: Table (

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

Parasite	Patients (n:140)		Controls (70)	
	(n)	(%)	(n)	(%)
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

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

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I	II	III
Mild patients (n 20)	Moderate patients (n: 60)	patients (n:60)

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 sig
NS: Non sngifi

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

[Note: F © 2021 Global Journals Parasitic Infection as a Risk Factor for Childhood Asthma in Upper Egypt Moderate Severe 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]

Figure 3: Table (

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Figure 4: Table (

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		I	II	III	P. value		
		Patients with +ve Ascaris infection by serology (n : 26)	patients with -ve Ascaris infection by serology (n:114)	Controls (n : 70)	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 A.E.C: Absolute Eosinophilic Count HS: Highly significant (P<0.
FEV1: Forced Expiratory Volume in 1 second ABG: Arterial Blood Gases NS: Non sngificant (P>0.05)
IL-5: Interleukin-5 S: Significant (P<0.05) MS: Moderately significant (P<
LTE4: Leukotriene E4

Figure 5: Table (4

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

(n:114)

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(mean ± SD)

PEFR: Peak Expiratory Flow Rate

FEV1: Forced Expiratory Volume 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

HS: Highly significant (P<0..001)

NS: Non sngificant (P>0.05)

Figure 6: Table (5

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) 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.

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