

Effect of *Ascaris lumbricoides* infection on T helper cell type 2 in rural Egyptian children

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Abstract: *Ascaris lumbricoides* is a neglected parasite that induces changes in host immune response. This study is conducted to define the serum levels of tumor necrosis factor alpha (TNF- α), interleukin-4 (IL-4), and interleukin-5 (IL-5) in some Egyptian children and their relations to intensity of infection, age, and ascariasis symptoms. Stool samples were examined using formol-ether concentration and Kato-Katz thick smear techniques. Sera of 60 *A. lumbricoides*-infected children and 20 controls were tested by enzyme-linked immunosorbent assay. The mean sera concentrations of TNF- α , IL-4, and IL-5 were 7.41 ± 2.5 pg/mL, 107.60 ± 18.3 pg/mL, and 389.52 ± 28.0 pg/mL, respectively. The controls had mean serum TNF- α 7.10 ± 2.4 pg/mL, IL-4 25.49 ± 2.6 pg/mL, and IL-5 88.76 ± 22.7 pg/mL. The difference in the concentration of sera cytokines was statistically significant for IL-4 and IL-5 ($P < 0.01$) between *A. lumbricoides*-infected children and controls. The intensity of infection correlated positively with IL-4 and IL-5 at $r = 0.959$ and $r = 0.919$, respectively. The concentrations of IL-4 and IL-5 correlated positively with the age at $r = 0.845$ and $r = 0.934$, respectively. Asthma and gastrointestinal tract upsets were correlated positively with IL-4 and IL-5. These data indicate that *A. lumbricoides* infection in our locality is associated with significantly high levels of IL-4 and IL-5.

Keywords: *Ascaris lumbricoides*, children, rural, Egypt, IL-4, IL-5, TNF- α

Introduction

Infection with gastrointestinal parasites has been globally detected as a serious public health problem in the tropical countries of the world.^{1,2} The large roundworm *Ascaris lumbricoides* is a ubiquitous pathogen of humans with worldwide distribution. It is estimated that ~1.5 billion infections are caused by *A. lumbricoides* alone.³ Such infections are associated with significant morbidity.⁴

It is reported that the prevalence of soil-transmitted helminth (STH) parasites is commonly high in conditions of poor hygiene and sanitation with more affection in children than adults.⁵ The mammalian host is infected with *A. lumbricoides* orally by ingestion of infective ova.^{6,7} Ascariasis is common in areas where defecation occurs in open latrines and stool is disposed arbitrarily in the surrounding environment.⁴ Therefore, contamination of food and water is so easy, the most common sources of infection.⁸

Human infection with *A. lumbricoides* has been associated with eosinophilia, fever, dyspnea, and cough what is known as Loeffler's syndrome during the larval migratory phase through the lung and abdominal pain during the intestinal phase.⁹ Ascariasis is characterized by eosinophilia, mast cell hyperplasia, and high levels of circulating immunoglobulin E (IgE).¹⁰

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On the one hand, numerous experimental animal studies have detected the significance of T helper cell type 2 responses to helminths of gastrointestinal tract and the prominence of certain interleukins (ILs), including IL-4, IL-5, IL-9, and IL-13, in facilitating expulsion of parasite and infection resistance.^{11,12} On the other hand, few studies have examined human cytokine responses to gastrointestinal helminths, especially those studies searching about cellular response to ascariasis in endemic population.^{13,14}

It should be remembered that individuals living in endemic areas are often infected with more than one STH parasite and many children with *A. lumbricoides*, particularly those with high parasite burdens, have a high probability of recent or current STH coinfections. This makes it more difficult to attribute specific immunologic effects to ascariasis alone even when blood cells are stimulated with *Ascaris* antigens in vitro because of the high degree of immunological cross-reactivity between STH antigens.¹⁵

It is highlighted that the interaction between different cytokines in the immunity against parasites is not as easy as one could imagine and can differ according to variant situations, including infection duration, sites difference in the host and species, or strains difference of hosts and parasite.¹⁶

Therefore, we conducted this study to illustrate the immunological milieu of *A. lumbricoides* by studying the serum levels of some Th1 cytokines as tumor necrosis factor alpha (TNF- α) and Th2 cytokines as IL-4 and IL-5 in infected children living in endemic rural areas in Mansoura, Egypt, and to check whether there is any relations between these cytokines and various famous clinical symptoms of ascariasis, such as abdominal cramps, diarrhea, and atopy. The relationship of the intensity of infection, age, and the concentration of these cytokines was also highlighted.

Materials and methods

Study area

This study was carried out in slums and destitute poor villages of rural areas of Mansoura, Egypt, where generality of its inhabitants use open latrines and the stool is disposed arbitrarily in the surrounding environment because of the bad habit of children who indiscriminately defecate in their surrounding locality. The mean annual rainfall of the study area is 589–670 mm. The annual minimum and maximum temperatures are 6.5°C and 29.9°C, respectively.

Study design

A cross-sectional study was conducted over 3 months after obtaining ethical permission from Ministry of Health, Mansoura

City, Dakahlia Province, Egypt. A medical aid convoy had explained the objectives and all procedures to the children and their families for their agreement. A written consent had been taken from all guardians of participated children.

Subject sampling

This study was performed on 60 children infected with *A. lumbricoides* known by their positive stool analysis. Their ages were from 4 years to 15 years with mean age 8.53 ± 2.7 . The studied group was 45 males and 15 females. A control group was included with 20 healthy children; 15 were males and five were females with matched age and sex. The investigated children were subjected to the following. 1) Complete history, including age, sex, and residence. 2) Clinical history of abdominal cramps and diarrhea. Detailed atopic symptoms, including asthma and skin allergy, were identified using the International Study of Asthma and Allergies in Childhood questionnaire,¹⁷ which included ever asthma, ever wheezing, and current wheezing for asthma. With regard to skin allergy, itchy rash ever, eczema ever, and itchy rash in the past year were identified. Ever asthma was defined as “asthma symptoms at any point in the past”. Ever wheezing was defined as “wheezing symptoms at any point in the past”. Current wheezing was defined as “wheezing symptoms within the last 12 months”. 3) Stool samples were collected, processed, and examined using direct saline, iodine, and formal ether concentration techniques. Infection intensity was detected by Kato-Katz thick smear technique as mentioned by Montresor et al¹⁸ to count the number of eggs per gram (epg) of feces according to World Health Organization standard, which divides the intensity of infection to light infection with 1–4.999 epg, moderate infection with 5,000–49,999 epg, and heavy infection with $\geq 50,000$ epg. 4) Sera were obtained from blood samples and subjected to enzyme-linked immunosorbent assay (ELISA) for the analysis of TNF- α (Quantikine® ELISA Human TNF- α Immunoassay; R&D Systems, Inc., Minneapolis, MN, USA), IL-4 (Quantikine® ELISA Human IL-4 Immunoassay; R&D Systems, Inc.), and IL-5 (Human Interleukin-5 ELISA; BioVendor – Laboratori medicina a.s., European Union: BioVendor GmbH) according to the manufacturer’s instructions.

Data management and statistical analysis

Entry of raw data and handling were done using Microsoft Excel spread sheets program and then were transferred to SPSS Version 21 for analysis. Descriptive statistics were computed. The studied data were presented as mean $\pm$ standard deviation. Independent samples *t*-test was used to compare means.

Table 1 Relationship between the studied cytokines and infected children age groups (N=60)

Age group (years) (no infected)	Mean TNF- α (pg/mL)	Mean IL-4 (pg/mL)	Mean IL-5 (pg/mL)	Mean parasite load (epg)
Preschool age (1–6) (14)	6.78 $\pm$ 1.6	105.17 $\pm$ 21.8	368 $\pm$ 14.3	4,482
School age (7–15) (46)	8.04 $\pm$ 2.7	110.04 $\pm$ 17.3	411.04 $\pm$ 23	8,595
	P=0.04	P=0.45	P<0.0001	P<0.0001
Mean	7.41 $\pm$ 2.5	107.60 $\pm$ 18.3	389.52 $\pm$ 28	6,538

Abbreviations: IL, interleukin; TNF- α , tumor necrosis factor alpha; epg, egg gram per stool.

Pearson's correlation was used to evaluate the association between the studied parameters. Chi-square test for independence was used to discover whether there is a relationship between the nominal parameters. At 5% level of significance, P -value <0.05 was considered significant in all analysis.

Results

In this study, it was found that school-aged children between 7 years and 15 years had higher parasite load 8,595 epg than preschool-aged children between 1 year and 6 years who showed parasite load 4,482 epg. With regard to the cytokines profile, those children 7–15 years had higher serum IL-5 (411.04 $\pm$ 23.0 pg/mL) than preschool-aged children (368.00 $\pm$ 14.3 pg/mL) with high significant difference (P <0.01). Also, the mean serum level of TNF- α was significantly higher in school-aged children (8.04 $\pm$ 2.7 pg/mL) than preschool-aged children (6.78 $\pm$ 1.6 pg/mL; P =0.04). Although IL-4 showed no significant difference in the two age groups (P =0.45), its mean serum level was 105.17 $\pm$ 21.8 pg/mL in preschool-aged children and 110.04 $\pm$ 17.3 pg/mL in school-aged children, respectively. In all infected children, the mean concentrations of the TNF- α , IL-4, and IL-5 were 7.41 $\pm$ 2.5 pg/mL, 107.60 $\pm$ 18.3 pg/mL, and 389.52 $\pm$ 28.0 pg/mL, respectively, Table 1.

Mild infection was reported among 13 children (3,508 epg), while 47 of them had moderate infection (8,777 epg). The difference between the mean concentration of IL-4 of those with mild infection (82.56 $\pm$ 3.8 pg/mL) and moderate infection (116.19 $\pm$ 13.4 pg/mL) was statistically

significant (P <0.01). In addition, the mean concentration of IL-5 for those with mild infection was 380.95 $\pm$ 9.8 pg/mL, and this was statistically different from those with moderate infection of 406.54 $\pm$ 29.0 pg/mL (P <0.01). Additionally, the children with mild infection had a mean TNF- α concentration of 6.33 $\pm$ 1.3 pg/mL, while those with moderate infection had a mean concentration of 8.14 $\pm$ 2.7 pg/mL, and this difference was significant (P =0.002). Comparing the sera cytokines concentration of the *A. lumbricoides*-infected children with that of the control group, it was found that mean concentration levels of both IL-4 and IL-5 of the infected children had shooting levels of 107.60 $\pm$ 18.3 pg/mL and 389.52 $\pm$ 28.0 pg/mL, respectively, than control group children in whom the mean concentration levels of IL-4 and IL-5 were 25.49 $\pm$ 2.6 pg/mL and 88.76 $\pm$ 22.7 pg/mL, respectively, with a high statistically significant difference (P <0.01). Meanwhile, the difference for TNF- α was not significant (P =0.31), as shown in Table 2.

We studied the correlation between the cytokines and the clinical data of the studied groups. We found that the intensity of infection was strongly and significantly (P <0.01) positively correlated with IL-4 and IL-5 at r =0.959 and r =0.919, respectively (Figures 1 and 2), while it was not correlated with TNF- α (r =0.208; P =0.065). Additionally, there was significant positive correlation between IL-4, IL-5, and the clinical symptoms of patients as diarrhea at r =0.607, r =0.579, abdominal cramps at r =0.531, r =0.445, and atopy at r =0.696, r =0.678, respectively (P <0.01). In contrast, TNF- α was not correlated with abdominal cramps, while it was positively

Table 2 The studied cytokines of 60 *A. lumbricoides*-infected children and their control subjects according to the intensity of infection

Intensity of infection (epg)	Intensity of infection (epg) (no infected)	Mean TNF- α (pg/mL)	Mean IL-4 (pg/mL)	Mean IL-5 (pg/mL)
Mild (1–4,999)	13 (3,508)	6.33 $\pm$ 1.3	82.56 $\pm$ 3.8	380.95 $\pm$ 9.8
Moderate (5,000–49,999)	47 (8,777)	8.14 $\pm$ 2.7	116.19 $\pm$ 13.4	406.54 $\pm$ 29.0
		P=0.002	P<0.0001	P<0.0001
Control	20 (0)	7.10 $\pm$ 2.4	25.49 $\pm$ 2.6	88.76 $\pm$ 22.7
		P=0.31	P<0.0001	P<0.0001

Abbreviations: TNF- α , tumor necrosis factor alpha; IL, interleukin; epg, egg gram per stool.

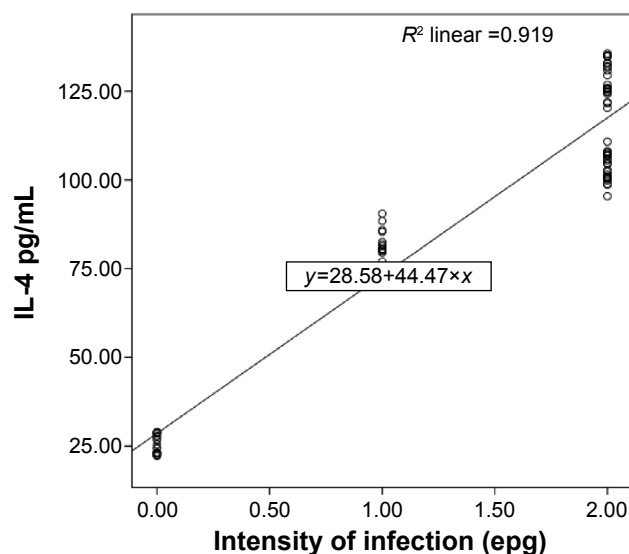


Figure 1 Correlation between the intensity of infection and IL-4.
Abbreviations: IL-4, interleukin-4; epg, egg gram per stool.

correlated with diarrhea and atopy at $r=0.320$ and $r=0.338$, respectively, as shown in Table 3. When we studied the associations of different degrees of atopy including that of asthma and eczema with the infection intensity of *A. lumbricoides*, we found significant associations ($P<0.01$ and $P=0.016$), respectively, as shown in Table 4.

Discussion

Parasitic helminths infestation is a common condition for billions of humans in different districts of the world where bad sanitation enhances infection and reinfection; children are notably susceptible to heavy infection when ill-health and poor growth result.¹⁹

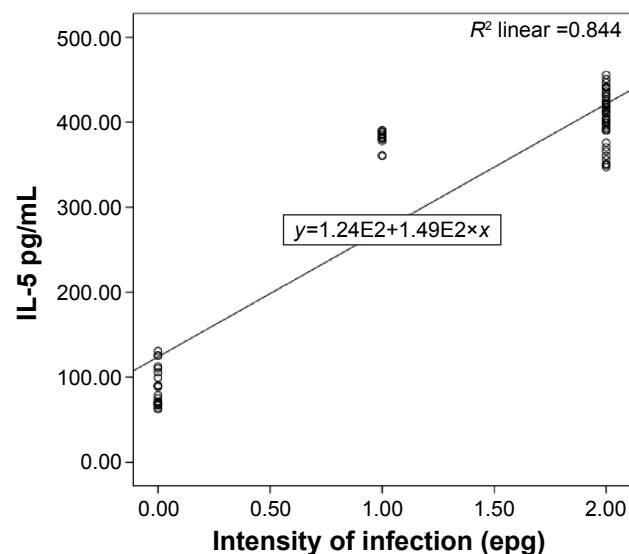


Figure 2 Correlation between the intensity of infection and IL-5.
Abbreviations: IL-5, interleukin-5; epg, egg gram per stool.

In the current study, we demonstrated that the school-aged children infected with *A. lumbricoides* produce higher levels of IL-4 and IL-5 than the control subjects. This observation was in agreement with Copper et al and Turner et al^{20,21} who revealed higher IL-4 profile among the examined subjects. This assertion is proved valid considering the important roles of IL-4 in helminthic infection. Not only IL-4 had a blocking effect on proinflammatory cytokines but also it is implicated in differentiation of B-cell and its activation with the antibodies release, which are specific to the antigen such as IgA and IgE, which could help in clearance of parasite.^{22,23} In contrast, Goddey et al²⁴ recorded low level of the anti-inflammatory IL-4 and they revealed that the low mean concentration of this IL could be responsible for maintenance of *A. lumbricoides* in their studied locality in Nigeria.

Moreover, our results with regard to IL-5 are proved valid, considering the major role of IL-5 in immunity against helminthic infection. IL-5 activates eosinophils. Antibodies may act in collaboration with cells such as macrophages and eosinophils by acting as a viaduct bringing the parasite and the activated cell together to assist the toxic molecules release directly onto the parasite surface, a process known as antibody-dependent cell-mediated cytotoxicity.²⁵ Also, the significantly high level of IL-5 concentration among the examined school children embroils IL-5 in immunopathogenesis of *A. lumbricoides* infection especially during the lung migratory phase. It has been documented that this phase associated clinically with *Ascaris* pneumonitis and accompanied by cellular infiltration, serous exudates, bronchial irritation and eosinophilia what is known as Loeffler's syndrome.^{26,27} The association between *A. lumbricoides* infection and asthma was initially proven valid by Lynch et al.²⁸

Th1 cells are known by their production of IL-2 and INF- γ . INF- γ stimulates resting macrophages and causing them to secrete an assortment of molecules, as TNF- α , reactive oxygen intermediates, and nitrous oxide. TNF- α has a cooperative effect with nitrous oxide in destroying the intracellular ingested parasites. These molecules, together with other things, intervene with DNA structure, mitochondrial function and membrane integrity and lead to the destruction of target cells. INF- γ has a negative effect on Th2 responses beside its positive role in stimulation of macrophages (Th1 response).²⁹

TNF- α is defined to be a key moderator of pathogenesis in a wide range of inflammatory, infectious, and autoimmune diseases.³⁰ TNF- α is a pleiotropic cytokine. It is necessary for homing of Th2 cells to the place of allergic inflammation. Actually, TNF- α has been exhibited to enhance Th2 cell-mediated phenomena in other systems, such as pathogenesis

Table 3 Correlation between the studied cytokines and both the intensity of infection and the clinical data of infected children

Variables	TNF- α		IL-4		IL-5	
	r	P-value	R	P-value	r	P-value
Diarrhea	0.320**	0.004	0.607**	<0.0001	0.579**	<0.0001
Abdominal cramps	-0.021	0.855	0.531**	<0.0001	0.445**	<0.0001
Atopy	0.338**	0.002	0.696**	<0.0001	0.678**	<0.0001
Parasite load	0.125	0.269	0.897**	<0.0001	0.836**	<0.0001
Intensity of infection	0.208	0.065	0.959**	<0.0001	0.919**	<0.0001

Notes: r denotes Pearson's correlation. P denotes significance. **Correlation is significant at the 0.01 level.

Abbreviations: TNF- α , tumor necrosis factor alpha; IL, interleukin.

of allergic inflammation in the gastric mucosa,³¹ and airway hyper responsiveness suggesting that TNF- α may also be important in mucosal Th2 responses during intestinal helminth infection.³²

In our study, although IL-4 and IL-5 levels were predominant, there was no impairment of TNF- α expression in the infected children in comparison to the control group which may explain a mixed Th1/Th2 response. It has been documented the critical role of TNF- α in regulation of Th2 cytokines.³³ This data deviates from the observation of Goddey et al²⁴ who revealed a significantly high TNF- α concentration among their investigated subjects. Meanwhile Geiger et al,⁹ recorded lower level of TNF- α in *Ascaris*-infected patients than in controls.

Previous studies have proved that in most of the gastrointestinal helminthic infections, the immune responses tend toward the Th2-like responses.^{34–36} Such responses are associated with significant production of IL-4, IL-5, IL-10, and IL-13 and with consequent strong IgE development, mast cell, and eosinophil responses.³⁷ The end result could be protective immunity. It has been revealed in earlier studies that adult and larval stage antigen of *A. lumbricoides* in human infection induces Th2 cytokine responses by the greater production of IL-4 in comparison to other cytokines such as IFN- γ .³⁸ This polarization toward Th2 cytokine responses

can be emphasized by our study as we reported significantly high level of IL-4 and IL-5 in comparison to TNF- α level production. In addition, we found that the concentration of IL-4 and IL-5 correlated positively with the age at $r=0.845$ and $r=0.934$, respectively. This is in agreement with the observation of Goddey et al.²⁴

In the current study, clinical symptoms related to gastrointestinal tract as abdominal cramps and diarrhea were highly and positively correlated with IL-4 and IL-5 altogether with parasitic load and intensity of infection, these findings were agreed by many researchers^{39,40} who stated that *Ascaris* infections are usually asymptomatic if infection involved only few worms, but as worm load increases, symptoms of gastrointestinal tract upsets develop. Despite the high prevalence of *Ascaris* infection in developing countries where diarrhea and other abdominal-related symptoms are dominating, in some infection there is alteration in *Ascaris*-related abdominal symptoms, which is explained by coinfection by other organisms that alter gastrointestinal tract milieu, leading to interference with penetration of the gut wall by *Ascaris* worms.⁴¹

On studying the relation of IL-4 and IL-5 to atopy in the studied groups, there was highly positive correlation between atopic symptoms, as asthma, eczema and the studied ILs. Prominent elements of Th2 immune response which is

Table 4 Associations of clinical types of atopy including asthma and eczema with demographic data and the degree of *Ascaris lumbricoides* infection using chi-square test

Parameters	Male, n (%)	Female, n (%)	P-value	Preschool age, n (%)	School age, n (%)	P-value	Mild infection, n (%)	Moderate infection, n (%)	P-value
No asthma	9 (15)	9 (15)	0.034	11 (18.3)	7 (11.6)	<0.0001	13 (21.6)	5 (8.3)	<0.0001
Ever asthma	16 (26.7)	3 (5)		2 (3.33)	17 (28.3)		0 (0)	19 (31.6)	
Ever wheezing	15 (25)	2 (3.33)		1 (1.66)	16 (26.6)		0 (0)	17 (28.3)	
Current wheezing	5 (8.3)	1 (1.66)	0.123	0 (0)	6 (10)	0.010	0 (0)	6 (10)	0.016
No eczema	28 (46.6)	9 (15)		14 (23.3)	23 (38.3)		13 (21.6)	24 (40)	
Itchy rash ever	6 (10)	0 (0)		0 (0)	6 (10)		0 (0)	6 (10)	
Eczema ever	6 (10)	1 (1.66)		0 (0)	7 (11.6)		0 (0)	7 (11.6)	
Itchy rash in past year	5 (8.3)	5 (8.3)		0 (0)	10 (16.6)		0 (0)	10 (16.6)	

Notes: P denotes significance <0.05.

evoked to promote worm expulsion from gut, simultaneously lead to high activity of mucosal eosinophils, mucus hyper secretion, and hyperactivity of mucosal muscles giving the full symptoms of asthma and explain the interaction between heavy parasitic infection and atopic symptoms. These relations cope with various studies.^{42–45}

It was stated that there is high risk of asthma due to sensitization to common aeroallergens, such risk is enhanced in *Ascaris*-infected kids reflexing the complex relationship between *Ascaris* and asthma susceptibility.⁴⁶

On the other hand, some researchers deny any association between *Ascaris* infection and atopy or other allergic disorders.^{47,48} Such finding might be explained by worm load and timing of infection, which may play role in the relationship between parasitic infection and the host immune response; few worms and/or adult age may protect the hosts against allergy.⁴⁹

Conclusion

In conclusion, the significantly high levels of IL-4 and IL-5 found in the sera of school-aged children from rural areas of Mansoura, Egypt, embarrasses these cytokines as the main mediators in the host immunity in response to infection with *A. lumbricoides* in our studied locality. However, further studies on cellular responses and other cytokine production, such as IL-9, IL-10, and IL-13, are needed to define another necessary parameters for obtaining protective immunity.

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Disclosure

The authors report no conflicts of interest in this work.

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