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Serum cytokines profiles in Nigerian children with *Ascaris lumbricoides* infection

Nmorsi Onyebiguwa Patrick Goddey*, Irior Dominic Osagie, Abu Maliki

Tropical Diseases Research Unit, Department of Zoology, Ambrose Alli University, Ekpoma, Nigeria

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ABSTRACT

Objective: To investigate the cytokines profile in the serum of some Nigerian children with *Ascaris lumbricoides (A. lumbricoides)* infection and relations between the intensity of infection, age and the concentration of these cytokines. **Methods:** Feacal samples from consenting children were examined using formol ether concentration techniques and Kato-Katz thick smear technique. Sera of 96 children comprising 76 *A. lumbricoides* infection and 20 controls were subjected to enzymes-linked immunosorbent assay. **Results:** The mean sera concentration of tumor necrosis factor alpha (TNF- α), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL-10) were (16.7±7.6) pg/mL, (4.6±0.8)pg/mL, (391.6±52.0) pg/mL and (181.2±30.4) pg/mL, respectively. The control subjects had the following mean serum cytokines: TNF- α (2.9±1.8) pg/m, IL-4 (4.0±0.1) pg/mL, IL-5 (125.1±50.1) pg/mL, IL-10 (74.5±54.2) pg/mL, respectively. The difference between the sera cytokines concentration of the *A. lumbricoides* infected children and their controls were statistically significant for TNF- α, IL- 5 and IL-10(χ^2= 9.99, P< 0.05; χ^2 = 137.24, P < 0.05; χ^2 = 44.30, P < 0.05, respectively). The intensity of infection correlated positively with TNF- α, and IL-5 (r = 0.93 and 0.98, respectively) while IL-4 and IL- 10 correlated negatively with the intensity of infection (r = -0.62 and 0.99, respectively). TNF- α, IL-4 and IL-5 correlated positively with age (r = 0.19, 0.33 and 0. 66, respectively). The mean cytokines between those with mild and moderate infections were statistically significant for TNF- α, IL-5 and IL-10 (χ^2 = 3.60, 4.52 and 5.30, respectively). The ratio of TNF- α : IL-10 was 0.092. **Conclusions:** Elevated IL-5, IL-10 and TNF- α found in the sera of the volunteers with Ascariasis implicates these cytokines as key mediators in the host responses to *A. lumbriocoides* infection in our studied area.

1. Introduction

Human gastrointestinal parasites have been generally recognized as a major public health problem in the tropic countries of the world. It is estimated that 1 billion people are infected world wide[1]. Geohelminths parasites are highly prevalent in conditions of poor sanitation and hygiene with children being more affected than adults[1,2]. Ascariasis caused by *Ascaris lumbricoides (A. lumbricoides)* is the most prevalent of the intestinal helminths that colonise the small intestine[3]. This infection is common in places where toilet facilities are inadequate and human feaces are disposed indiscriminately within our surrounding environment[4]. Here, food and water are easily contaminated which can subsequently constitute sources of infection. Ascariasis have been associated with eosinophilic pneumonia during the migratory phase through the lungs[5] as well as abdominal symptoms. Immune responses as expressed by production of cytokines in ascariasis have been documented globally[6-16]. Some of these investigations potentiate the relevance of interleukins in vaccine and chemotherapy[17,18]. There is dearth of information in this regard in this part of the globe as most data are epidemiological[2,19,20].

We therefore investigated and reported the cytokines profile in the serum of some Nigerian children with *A. lumbricoides* infection. The relationship of the intensity of infection, age and the concentration of these cytokines were highlighted.

2. Materials and methods

*Corresponding author: Prof. OPG Nmorsi, Tropical Diseases Research Unit, Department of Zoology, Ambrose Alli University, Ekpoma, Nigeria.
Tel: 234 (0) 8063309768
E-mail: nmorsiopg@yahoo.com

This survey was carried out in Igwe-Oke, Owan East Local Government Area of Edo State, Majority of the inhabitants use pit latrines while the children defecate indiscriminately in their neighbourhood.

Ethical permission was obtained from Edo State Ministry of Health, Benin City, Edo State, Nigeria. The villagers were mobilized by a community mobilization campaign where the objectives, all procedures were explained to the children and their parents for their consent. Feacal samples were obtained from the consenting children. The samples were processed and examined using formol ether concentration techniques. The intensity of infection was ascertained by counting the egg per gram of feaces according to WHO standard using the Kato-Katz thick smear technique as described by Montresor *et al*[21]. Sera were obtained from blood samples of 76 individuals with *A. lumbricoides* infection and 20 control subjects. The sera were immediately subjected to enzyme - linked immunosorbent assay for the analysis of interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL-10) and tumor necrosis factor-α (TNF-α) according to the manufacturer's instructions.

The data obtained from the investigation were subjected to statistical analysis namely chi-square and correlation using Microsoft Excel package.

3. Results

The cytokine profile of 76 *A. lumbricoides* infected volunteers with their age groups and intensity of infection and their control are presented in Table 1. The children between the age of 6 and 10 years had the highest level of parasite load (12 650 epg) while the least (2 010 epg) was observed among the children below 5 years of age. These children had the highest serum TNF-α [(25.1±4.1) pg/mL] and IL-5 [(430.4±150.7) pg/mL), respectively. The highest serum IL-4 [(4.9±3.1) pg/mL] and IL-10 [(212.8±81.1) pg/mL] were observed among the children below 5 years age. In all, the mean concentration of the TNF-α, IL-4, IL-5 and IL-10 were (16.7±7.6) pg/mL, (4.6±0.8) pg/mL, (391.6±52.0) pg/mL and (181.2±30.4) pg/mL, respectively. The relationship between intensity of infection and TNF-α, IL -4 and IL-5 were positively correlated at r = 0.19, 0.33, 0.66, respectively while the relationship between IL-10 and the intensity of infection was negatively correlated at r = - 0.46. The ratio of TNF-α /IL-10 was 0.092.

Moderate infection was reported among 44 children while 32 of them had mild infection. The difference between the mean TNF-α concentration of those with mild infection [(11.5±3.5)pg/mL] and moderate infection [(22.5±6.1)pg/mL] was statistically significant (χ^2 = 3.60; P< 0.05). The children with mild infection had a mean IL-4 concentration of (3.8±1.1) pg/mL while those with moderate infection had a mean concentration of (4.9±2.2) pg/mL and this difference was not significant (χ^2 = 0.02, P>0.05). The mean IL-5 concentration for those with mild infection was (361.0±117.2) pg/mL and this was statistically different from those with moderate infection [(420.5±73.3) pg/mL] (χ^2 = 4.52, P<0.05). The difference between the mean IL-10 concentration of the children with mild infection [(202.9±102.9)pg/mL] and those with moderate infection [(159.1±112.5)pg/mL] was statistically significant (χ^2 = 5.30, P< 0.05). Also the differences between the sera cytokines concentration of *A. lumbricoides* infected and their control children were statistically significant for TNF-α (χ^2 = 9.99, P<0.05), IL-5 (χ^2 = 137.24, P<0.05), and IL-10 (χ^2 = 44.30, P<0.05). The differences for the IL-4 was not significant (χ^2 = 0.02, P>0.05).

Table 1
The cytokines profile of 76 *A. lumbricordes* infected volunteers with their age groups.

Age groups in years Number infected	Mean parasite load (epg)	Mean TNF-α (pg/mL)	Mean IL-4 (pg/mL)	Mean IL-5 (pg/mL)	Mean IL-10 (pg/mL)
< 5	27 (2 010)	10.0±3.5	4.9±3.1	332.5±20.6	212.8±81.1
6-10	31 (12 650)	25.1±4.1	3.7±0.8	430.4±150.6	152.5±93.6
>10	18 (9 000)	15.4±4.5	5.2±2.5	411.9±135.0	177.5±51.9
Mean	(7 886.7)	16.7±7.6	4.6±0.8	391.6±52.0	181.2±30.4

Table 2
The cytokines profile of 76 *A. lumbricoides* infected volunteers and their control subjects according to the intensity of infection.

Intensity of infection mber of infected	Intensity of infection (epg)	Mean TNF-α (pg/mL)	Mean IL-4(pg/mL)	Mean IL-5 (pg/mL)	Mean IL-10 (pg/mL)
Mild(1-4 999)	32 (3 850)	11.5±3.5	3.8±1.1	361.0±117.2	202.9±102.9
Moderate (5 000-49 999)	44 (11 910)	22.5±6.1	4.9±6.1	420.5±73.3	159.1±112.5
Mean	(7 880)	17.0±7.8	4.4±0.8	390.8±43.1	181.0±31.0
Control	20 (0)	2.9±1.8	4.0±0.1	125.0±50.1	74.5±54.2