

**Original Article****Open Access****Comparative analysis of poliovirus-specific IgA and cytokine levels in the sera of *Ascaris lumbricoides*-infected and helminth-negative Nigerian children after oral poliovirus vaccination**

Akinwande, K. S., and \*Arinola, G. O.

Department of Immunology, University of Ibadan, Nigeria

\*Correspondence to: [drarinolaog64@yahoo.com](mailto:drarinolaog64@yahoo.com); +234 80 23451520**Abstract:**

**Background:** Intestinal helminth infection is associated with altered immune responses and compromised vaccine efficacy in infected children. Altered immune response due to *Ascaris lumbricoides* infection may compromise efficacy of oral poliovirus vaccination in children. There is no information on humoral immune response during oral poliovirus (OP) vaccination of *A. lumbricoides*-infected Nigerian children. The objective of this study is to determine the serum levels of cytokines (tumour necrosis factor- $\alpha$  TNF- $\alpha$ , interferon- $\gamma$  IFN- $\gamma$ , interleukins -4, -6, -8, -10) and poliovirus-specific IgA (PV-IgA) antibody in children infected with *A. lumbricoides* compared with helminth-negative children (control) before and after oral poliovirus vaccination.

**Methodology:** Twenty-three *A. lumbricoides*-infected children between ages 5-15 years (13 males and 10 females) and 23 age (4-15 years) and sex-matched helminth-negative children who met selection criteria were enrolled into the study after ethical approval and informed consent. Their stool samples were examined for helminth ova using concentration technique. Sera were collected before and 3 weeks after OP vaccinations, and serum concentrations of IFN- $\gamma$ , TNF- $\alpha$ , IL-4, -6, -8, -10, and poliovirus-specific IgA concentrations were determined by enzyme-linked immunosorbent assay. The level of statistical significance was set at  $\alpha_{0.05}$ .

**Results:** Pre-vaccination serum levels of IFN- $\gamma$ , IL-4, IL-6 and IL-8 were significantly higher in *A. lumbricoides*-infected children compared with pre-vaccination levels in helminth-negative children. Post-vaccination serum levels of IFN- $\gamma$ , IL-4 and IL-8 were significantly higher in *A. lumbricoides*-infected children compared with post-vaccination serum levels in helminth-negative children. In the *A. lumbricoides*-infected children, pre-vaccination serum levels of IL-6 and IL-8 were significantly higher compared with post vaccination levels while pre-vaccination serum levels of IFN- $\gamma$ , IL-4 and IL-8 were significantly higher in helminth-negative children compared with the post-vaccination levels. There was no significant reduction in post-vaccination median serum level of PV-IgA compared with level before vaccination in *A. lumbricoides*-infected children. Also, there was no significant increase in post-vaccination median serum level of PV-IgA compared with level before vaccination in helminth-negative children.

**Conclusion:** Oral polio vaccine administration caused decrease expression of inflammatory cytokines (IL-6 and IL-8) in *A. lumbricoides*-infected school children, and *A. lumbricoides* infection may reduce PV-IgA production following OP vaccination.

**Keywords:** *Ascaris lumbricoides* infection, cytokines, children, poliovirus vaccination

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**Analyse comparative des taux d'IgA et de cytokines spécifiques du poliovirus dans les sérums d'enfants Nigériens infectés par *Ascaris lumbricoides* et négatifs pour les helminthes après vaccination orale contre le poliovirus**

Akinwande, K. S., et \*Arinola, G. O.

Département d'immunologie, Université d'Ibadan, Nigéria

\*Correspondance à: [drarinolaog64@yahoo.com](mailto:drarinolaog64@yahoo.com); +234 80 23451520

## Abstrait:

**Contexte:** L'infection intestinale par les helminthes est associée à des réponses immunitaires modifiées et à une efficacité du vaccin compromise chez les enfants infectés. Une réponse immunitaire modifiée due à une infection à *Ascaris lumbricoides* peut compromettre l'efficacité de la vaccination antipoliomyélitique orale chez les enfants. Il n'y a pas d'informations sur la réponse immunitaire humorale lors de la vaccination antipoliomyélitique orale (OP) des enfants nigériens infectés par *A. lumbricoides*. L'objectif de cette étude est de déterminer les taux sériques de cytokines (facteur de nécrose tumorale- $\alpha$  TNF- $\alpha$ , interféron-gamma IFN- $\gamma$ , interleukines -4, -6, -8, -10) et IgA spécifiques du poliovirus (PV-IgA) chez les enfants infectés par *A. lumbricoides* par rapport aux enfants anti-helminthes (témoin) avant et après la vaccination antipoliomyélitique orale.

**Méthodologie:** Vingt-trois enfants infectés par *A. lumbricoides* âgés de 5 à 15 ans (13 hommes et 10 femmes) et 23 enfants (4 à 15 ans) et enfants de sexe masculin négatifs pour les helminthes qui répondaient aux critères de sélection ont été inclus dans l'étude après approbation éthique et consentement éclairé. Leurs échantillons de selles ont été examinés pour les ovules d'helminthes en utilisant une technique de concentration. Les sérums ont été collectés avant et 3 semaines après les vaccinations OP, et les concentrations sériques d'IFN- $\gamma$ , de TNF- $\alpha$ , d'IL-4, -6, -8, -10 et d'IgA spécifiques du poliovirus ont été déterminées par un test d'immunosorbant lié à une enzyme. Le niveau de signification statistique a été fixé à  $\alpha 0,05$ .

**Résultats:** Les taux sériques d'IFN- $\gamma$ , d'IL-4, d'IL-6 et d'IL-8 avant la vaccination étaient significativement plus élevés chez les enfants infectés par *A. lumbricoides* par rapport aux niveaux avant la vaccination chez les enfants négatifs pour les helminthes. Les taux sériques d'IFN- $\gamma$ , d'IL-4 et d'IL-8 après la vaccination étaient significativement plus élevés chez les enfants infectés par *A. lumbricoides* par rapport aux taux sériques après vaccination chez les enfants négatifs pour les helminthes. Chez les enfants infectés par *A. lumbricoides*, les taux sériques d'IL-6 et d'IL-8 avant la vaccination étaient significativement plus élevés par rapport aux niveaux après vaccination, tandis que les taux sériques d'IFN- $\gamma$ , d'IL-4 et d'IL-8 avant la vaccination étaient significativement plus élevés chez les enfants négatifs aux helminthes par rapport aux niveaux post-vaccination. Il n'y a pas eu de réduction significative du taux sérique médian de PV-IgA après la vaccination par rapport au niveau avant la vaccination chez les enfants infectés par *A. lumbricoides*. En outre, il n'y avait pas d'augmentation significative du taux sérique médian de PV-IgA après la vaccination par rapport au niveau avant la vaccination chez les enfants négatifs pour les helminthes.

**Conclusion:** L'administration du vaccin antipoliomyélitique oral a entraîné une diminution de l'expression des cytokines inflammatoires (IL-6 et IL-8) chez les écoliers infectés par *A. lumbricoides*, et l'infection par *A. lumbricoides* peut réduire la production de PV-IgA après la vaccination OP.

**Mots clés:** infection à *Ascaris lumbricoides*, cytokines, enfants, vaccination contre le poliovirus

## Introduction:

*Ascaris lumbricoides* (also known as roundworms) belongs to a group of intestinal parasitic worms known as soil-transmitted helminths (STH) (1). STH infects over one and half billion people worldwide which correspond to about 24% of population of the world with very high prevalence in China, the Americas, sub-Saharan Africa and East Asia (1). It was approximated that *A. lumbricoides* alone infects about 819 million people worldwide (2). In Nigeria, it is the commonest among the STH infection that affect children who usually require extensive vaccination (3). Morbidity is related to the worm intensity. Minor infection with the parasite often gives no symptoms but heavy infection may result in symptoms such as passing of worms in faeces, abdominal discomfort, intestinal ulceration, cough, bloody sputum and fever (4).

*Ascaris lumbricoides* infection is associated with mast cells hyperplasia, eosinophilia and high levels of circulating immunoglobulin E which confers protective immune response against the invading larvae, but may also be a marker of enhanced type-2 immune response (5,6). Some studies have also demonstrated marked Th-2 cytokine responses in *A. lumbricoides* infection and the roles of IL-4, IL-5, IL-9 and IL-13-

associated pathways in the mediation of resistance to the infection and the expulsion of the helminthic parasite (7).

Polioviruses are RNA viruses that colonize the gastrointestinal tract particularly the intestine and oropharynx. They infect humans alone and are of three serotypes; poliovirus type 1 (PV1), type 2 (PV2), and type 3 (PV3), with slight differences between each based on the make-up of their capsid protein (8). Vaccination against poliovirus infection came about in 1955 with the introduction of live-attenuated oral polio vaccine (OPV) (Sabin types 1, 2 and 3) and 1961, with inactivated (killed) polio vaccine (IPV). OPV has however been the choice vaccine for the global eradication programme based on its action on mucosal immunity, very low cost, and the ease of oral administration of the vaccine.

OPV produces antibodies to all the three poliovirus strains in the blood and protect individuals against nerve paralysis if infected with poliovirus by preventing the spread of the virus to the nervous system but also protect individuals from being infected with poliovirus by producing local immune response in the mucous lining of the intestine, which is the primary poliovirus multiplication site (9). Vaccination against poliovirus induces strong Th-1 response (IL-2

and IFN- $\gamma$ ) which confers protective immune response in vaccinated hosts (10). However, helminth infection may affect responses to vaccine through the expression of regulatory cytokines (IL-10) or cause Th-1 to Th-2 shift, which down-regulates the expression of Th-1 cytokines following vaccination (11). Studies that investigate factors influencing the efficacy of OP vaccines among Nigerian children are sparse and are therefore necessary.

Low immunogenic responses to routine vaccinations have been reported among populations in low-income-countries when compared with those in the developed countries (12,13). Several factors such as maternal trans-placental antibody titres, micronutrient malnutrition, breast-feeding practices, stomach acidity and interfering gut flora have been reported to be responsible for these observations (14). STH infections have generally been proposed to contribute to malnutrition and low intelligent quotient in children, as a result of reduction in digestion and absorption, helminth induced chronic inflammation, and loss of nutrients (15). Unfortunately, little attention has been paid to the possible effect of STH, especially *A. lumbricoides* infection on vaccine efficacy, specific micronutrients levels or specific vaccine immune factors. There is therefore the need to assess vaccine-specific immune status of Nigerian children in whom vaccine administration is compulsory and helminth infection is common. This comparative study determined the serum poliovirus-specific IgA antibody and cytokine levels (TNF- $\alpha$ , IFN- $\gamma$ , IL-4, IL-6, IL-8, and IL-10) in *Ascaris lumbricoides*-infected and helminth-negative school-aged children before and three weeks after oral poliovirus vaccinations.

## Materials and method:

### Study setting and participants

The study center, participant selection, collection and examination of stool have earlier been reported (6). Briefly, of 349 pre-school and school aged children who were screened for intestinal helminth infection, 23 children age 5–15 years, whose stool microscopic examination revealed only *A. lumbricoides*, and met other inclusion criteria were selected as the study subjects. Twenty-three gender matched children age 4–15 years, whose stool sample revealed no eggs or larvae of any stool helminth were selected as controls and regarded as helminth-negative.

All study participants were apparently healthy and with no sign of any infection. The parents claimed that the children received oral polio vaccines at infancy. Any child on medication, with malaria parasites and whose

parent refused participation were excluded from the study.

### Ethical consideration

Ethical clearance was obtained from the University of Ibadan/University College Hospital Joint Ethics Committee (UI/EC/13/0331) and Oyo State Ministry of Health (AD/13/479/517). Participants were enrolled following town-hall and the parents-teachers association's health awareness meetings carried out in the communities and schools respectively. Children whose parents consented to participate in the study were recruited. A general de-worming exercise was carried out at the end of the exercise.

### Stool specimen collection and processing

Faecal specimen was scooped using spatula into a labeled screw cap polystyrene bottle and tightly screwed. The stool specimens were examined microscopically within 12 hours of collection using the formol-ether concentration technique for helminth identification (16). The magnifications of x10 and x40 objectives of the light microscope were used to identify characteristic ova of the intestinal helminth.

### Blood sample collection and processing

Blood sample collection was carried out before vaccination and three weeks after vaccination from each child. At each instance, five millilitres of venous blood was obtained from the antecubital vein and dispensed into plain polystyrene bottle. The blood samples were allowed to clot, and the clotted samples retracted and spun at 3000rpm for 10 min. The sera were removed into plain sterile cryo-precipitate tube and frozen at -20°C until analysis.

### Procedure for oral poliovirus vaccination

The oral poliovirus vaccine (Sabin, GlaxoSmithkline) was supplied in glass vials with dropper and stored at 4°C inside a thermos flask. It was allowed to attain normal temperature before administration. The child's mouth was opened gently between fingers to make the child's lips point outward. The dropper was held over the child's mouth at an angle of about 45 degrees and two drops of the vaccine was dropped onto the rear part of the child's tongue. Each child was allowed to resume normal sitting position observed for about 10 minutes after the administration of the vaccine, to ensure it was not vomited.

### Laboratory estimation of cytokines and IgA by ELISA

The serum levels of IFN- $\gamma$ , TNF- $\alpha$ , IL-4, IL-8, IL-6, IL-10 and PV-specific IgA

were determined using enzyme linked immunosorbent assay (ELISA) as described by the manufacturers (Abcam, MA, USA; AssayPro, MO, USA; Calbiotech, USA, and Sunlong Biotech, Hangzou, China) and as previously carried out (6). The ELISA was based on direct antigen-antibody interaction. Protein antigens present in patient's sample were allowed to bind in wells of plate pre-coated with antibodies. The plate was washed after a period of incubation to remove the remaining sample components and reduce interference. To this plate, a corresponding second enzyme-linked antibody was added, which catalyzed the conversion of a suitable substrate to produce a colour reaction. The colour produced was measured as a function of antigens present in the sample.

**Statistical analysis**

The data generated were expressed as median (interquartile range) and represented as figures in percentages and tables. Statistical data evaluation was carried out using the Statistical Package for the Social Sciences (SPSS) version 21.0. The Mann-Whitney U test was used to compare differences in levels of serum cytokines between *Ascaris lumbricoides* infected and helminth-negative subjects. Wilcoxon Signed Ranks test was used to compare pre- and post-vaccinated cytokines levels in both groups. The level of statistical significance was set at  $\alpha_{0.05}$ .

**Results:**

Table 1 shows the serum cytokine levels of *A. lumbricoides*-infected children compared with helminth-negative children

before OP vaccination. Serum levels of IFN- $\gamma$  (113.41 [IQR 68.41-146.52] vs 67.21 [IQR 23.46-93.29]pg/ml,  $p=0.014$ ), interleukin-4 (191.2 [IQR 127.9-320.6] vs 92.7 [IQR 66.8-151.1]pg/ml, $p=0.001$ ), interleukin-8 (1211.1 [IQR 696.4-1226.7] vs 778.4 [IQR 232.9-899.9]pg/ml,  $p=0.014$ ) and interleukin-6 (13.12 [IQR 9.37-22.15] vs 5.54 [IQR 3.02-7.29] pg/ml,  $p=0.000$ ) were significantly higher in *A. lumbricoides*-infected children compared with helminth-negative children. The serum levels of TNF- $\alpha$  (50.90 [IQR 40.41-69.34] vs 40.09 [IQR 32.97 - 58.30] pg/ml,  $p=0.145$ ) and IL-10 (0.11 [IQR 0.05-0.61] vs 0.12 [IQR 0.06 - 0.43]ng/ml,  $p=0.720$ ) in *A. lumbricoides*-infected children compared with helminth-negative children were not statistically significant.

Table 2 shows the serum cytokine levels in *A. lumbricoides*-infected children compared with helminth-negative children after oral polio vaccination. Post vaccination serum levels of IFN- $\gamma$  (96.23 [IQR 74.83-123.29] vs 25.86 [IQR 17.01-30.57]pg/ml,  $p=0.000$ ), IL-4 (170.8 [IQR 133.0-199.3] vs 41.1 [IQR 31.2-64.4]pg/ml,  $p=0.000$ ) and IL-8 (805.6 [IQR 603.2-821.4] vs 233.4 [IQR 205.0-251.7]pg/ml,  $p=0.000$ ) were significantly higher in *A. lumbricoides*-infected children compared with post-vaccination levels in helminth-negative children. The post-vaccination serum levels of TNF- $\alpha$  (44.19 [IQR 35.41 - 54.59] vs 33.53 [IQR 29.12-51.56]pg/ml,  $p=0.063$ ), IL-6 (7.58 [6.01-10.55] vs 6.33 [IQR 3.87 - 8.17]pg/ml,  $p=0.174$ ) and IL-10 (0.08 [IQR 0.06-0.21] vs 0.09 [IQR 0.08-0.19]ng/ml,  $p=0.800$ ) in *A. lumbricoides*-infected children compared with helminth-negative children were not statistically significant.

Table 1: Serum cytokine levels *Ascaris lumbricoides* -infected children compared with helminth-negative children before oral polio vaccination

Serum values	<i>A. lumbricoides</i> -infected (n=23)	Helminth - negative (n=23)	U	p-value
IFN- $\gamma$ (pg/ml)	113.81 (68.41-146.52)	67.21 (23.46-93.29)	36.000	0.014*
TNF- $\alpha$ (pg/ml)	50.90 (40.41-69.34)	40.09 (32.97-58.30)	56.000	0.145
IL-4 (pg/ml)	191.2 (127.9-320.6)	92.7 (66.8-151.1)	21.000	0.001*
IL-10 (ng/ml)	0.11 (0.05-0.61)	0.12 (0.06-0.43)	78.000	0.720
IL-8 (pg/ml)	1211.1 (696.4-1226.7)	778.4 (232.9-899.9)	36.000	0.014*
IL-6 (pg/ml)	13.12 (9.37-22.15)	5.54 (3.02-7.29)	12.000	0.000*

\*Significant at  $\alpha_{0.05}$ ; U - Mann Whitney U Test; *A. lumbricoides* – *Ascaris lumbricoides*

Table 2: Serum cytokine levels in *Ascaris lumbricoides* -infected children compared with helminth-negative children after oral polio vaccination

Serum values	<i>A. lumbricoides</i> -infected (n=23)	Helminth -negative (n=23)	U	p-value
IFN-γ (pg/ml)	96.23 (74.83-123.29)	25.86 (17.01-30.57)	0.000	0.000*
TNF-α (pg/ml)	44.19 (35.41-54.59)	33.53 (29.12-51.56)	48.000	0.063
IL-4 (pg/ml)	170.8 (133.0-199.3)	41.1 (31.2 - 64.4)	4.000	0.000*
IL-10 (ng/ml)	0.08 (0.06-0.21)	0.09 (0.08-0.19)	80.000	0.800
IL-8 (pg/ml)	805.6 (603.2-821.4)	233.4 (205.0-251.7)	6.000	0.000*
IL-6 (pg/ml)	7.58 (6.01-10.55)	6.33 (3.87-8.17)	58.000	0.174

\*Significant at α0.05; U - Mann Whitney U Test; *A. lumbricoides* - *Ascaris lumbricoides*

Table 3: Serum cytokine levels in *Ascaris lumbricoides*-infected school children before and after oral polio vaccination

Serum values	Pre-vaccination (n=23)	Post-vaccination (n=23)	Z	p-value
IFN-γ (pg/ml)	113.81 (68.41-146.52)	96.23 (74.83-123.29)	0.260	0.795
TNF-α (pg/ml)	50.90 (40.41-69.34)	44.19 (35.41-54.59)	0.876	0.381
IL-4 (pg/ml)	191.2 (127.9-320.6)	170.8 (133.0-199.3)	1.444	0.149
IL-10 (ng/ml)	0.11 (0.05-0.61)	0.08 (0.06-0.21)	1.642	0.101
IL-8 (pg/ml)	1211.1 (696.4-1226.7)	805.6 (603.2-821.4)	2.208	0.027*
IL-6 (pg/ml)	13.12 (9.37-22.15)	7.58 (6.01-10.55)	3.011	0.003*

\*Significant at α0.05; Z - Wilcoxon Signed Ranks Test

Table 3 shows the serum cytokine levels in *A. lumbricoides*-infected children before and after oral polio vaccination. Pre-vaccination serum levels of IL-8 (1211.1 [IQR 696.4 - 1226.7] vs 805.6 [IQR 603.2-821.4]pg/ml, p=0.027) and IL-6 (13.12 [IQR 9.37-22.15] vs 7.58 [IQR 6.01-10.55]pg/ml, p=0.000) were significantly higher compared with post-vaccination levels. The pre-vaccination serum levels of IFN-γ (113.81 [IQR 68.41-146.52] vs 96.23 [IQR 74.83-123.29] pg/ml, p=0.795), TNF-α (50.90 [IQR 40.41-69.34] vs 44.19 [IQR 35.41-54.59]pg/ml, p=0.381), IL-4 (191.2 [IQR 127.9-320.6] vs 170.8 [IQR 133.0-199.3]pg/ml, p=0.149) and IL-10 (0.11 [IQR 0.05-0.61] vs 0.08 [IQR 0.06-0.21]ng/ml, p=0.101) compared with post vaccination levels were not statistically significant.

Table 4 shows the serum cytokine levels in helminth-negative children before and after oral poliovirus vaccination. Pre-vaccination serum level of IFN-γ (67.21[IQR 23.46-93.29] vs 25.86 [IQR 17.01-30.57] pg/ml, p=0.037), IL-4 (92.7 [IQR 66.8-151.1] vs 41.1 [IQR 31.2-64.4]pg/ml, p=0.013) and IL-8 (778.4 [IQR 232.9-899.9] vs 233.4 [IQR 205.0-251.7]pg/ml, p=0.012) were significantly higher compared with post-vaccination levels. The pre-vaccination serum levels of TNF-α (40.09 [IQR 32.97-58.30] vs 33.53 [IQR 29.12-51.56]pg/ml, p=0.114), IL-10 (0.12 [IQR 0.06-0.43] vs 0.09 [IQR 0.08-0.19]ng/ml, p=0.201), and IL-6 (5.54 [IQR 3.02-7.29] vs 6.33 [IQR 3.87-8.17] pg/ml, p=0.241) compared with post vaccination levels were not statistically significant.

Table 4: Serum cytokine levels in helminth uninfected school children before and after oral polio vaccination

	Pre-vaccinated (n=23)	Post-vaccinated (n=23)	Z	p-value
IFN-γ (pg/ml)	67.21 (23.46-93.29)	25.86 (17.01-30.57)	2.090	0.037*
TNF-α (pg/ml)	40.09 (32.97-58.30)	33.53 (29.12-51.56)	1.580	0.114
IL-4 (pg/ml)	92.7 (66.8-151.1)	41.1 (31.2 - 64.4)	2.497	0.013*
IL-10 (ng/ml)	0.12 (0.06-0.43)	0.09 (0.08-0.19)	1.279	0.201
IL-8 (pg/ml)	778.4 (232.9-899.9)	233.4 (205.0-251.7)	2.505	0.012*
IL-6 (pg/ml)	5.54 (3.02-7.29)	6.33 (3.87-8.17)	1.174	0.241

\*Significant at α<sub>0.05</sub>; Z - Wilcoxon Signed Ranks Test

Table 5: Serum levels of Poliovirus-Specific IgA in school aged children with and without helminth *Ascaris lumbricoides* before and after oral polio vaccination

	Poliovirus specific -IgA (U/ml)
Pre-vaccination	
<i>A. lumbricoides</i> -positive(n=23)	1.831 (1.609 - 2.575)
Helminth-negative (n=23)	1.983 (1.368 - 4.234)
Post-vaccination	
<i>A. lumbricoides</i> -positive(n=23)	1.782 (1.381 - 2.979)
Helminth-negative (n=23)	2.488 (1.597 - 3.641)
Z, p-value <sup>a</sup>	0.213, 0.831
Z, p-value <sup>b</sup>	0.153, 0.878
U, p-value <sup>c</sup>	0.025, 0.980
U, p-value <sup>d</sup>	0.209, 0.223

\*Significant at α<sub>0.05</sub>; <sup>a</sup>*A. lumbricoides*-positive pre-vaccination vs *A. lumbricoides*-positive post-vaccination; <sup>b</sup>Helminth-negative pre-vaccination vs Helminth-negative post-vaccination; <sup>c</sup>*A. lumbricoides*-positive pre-vaccination vs Helminth-negative pre-vaccination; <sup>d</sup>*A. lumbricoides*-positive post-vaccination vs Helminth-negative post-vaccination; U=Mann Whitney U Test; Z=Wilcoxon Signed Ranks Test

Table 5 shows the serum levels of poliovirus-specific IgA (PV-IgA) in *A. lumbricoides*-infected and helminth-negative children before and after oral poliovirus vaccination. The post-vaccination median serum level of PV-IgA compared with pre-vaccination level in *A. lumbricoides*-infected children (1.782 [1.381-2.979] vs 1.831 [1.609-2.575] U/ml) was not statistically significant (p=0.831). Similarly, the post-vaccination median serum level of PV-IgA compared with pre-vaccination serum level in helminth-negative children (2.488 [1.597-3.641] vs 1.983 [1.368-4.234] U/ml) was not statistically significant (p=0.878). Also, post-vaccination median serum level of PV-IgA in *A. lumbricoides*-infected children compared with post vaccination serum level in helminth negative children (1.782 [1.381-2.979] vs 2.488 [1.597-3.641] U/ml) was not statistically significant (p=0.233). There was also

no significant difference (p=0.980) in serum PV-IgA levels of helminth-negative children compared with *Ascaris lumbricoides*-infected school aged children (1.983 [1.368-4.234] vs 1.831 [1.609-2.575] U/ml).

### Discussion:

Global efforts at eradicating poliomyelitis through vaccination has recorded successes but not without challenges. The dramatic progress in reducing the virus incidence had been made as at year 2000 (17) but polio has remained endemic in few countries (8,17). Failure of the oral poliovirus vaccine and the emergence of circulating vaccine-derived polioviruses (cvPV) are part of the main challenges facing global eradication of the disease (18). While several reasons and solutions have been postulated as means of improving poliovirus immuni-

zation outcome, studies that investigate the immuno-modulatory effects of intestinal helminth infection as possible reason for failure of OPV in the affected countries were not encountered. Our study therefore focused on the dynamics of the interplay between pro-inflammatory and anti-inflammatory cytokine responses following vaccination, which conversely may give insight into the effectiveness of OPV in *Ascaris lumbricoides*-infected Nigerian children.

Significantly higher serum levels of IFN- $\gamma$  and IL-4 were observed in *Ascaris lumbricoides*-infected children compared to helminth-negative children before vaccination (Table 1) and after vaccination (Table 2). IFN- $\gamma$  is a cytokine that is critical for the innate and adaptive immunity against viral, some bacterial and protozoal infections. It is produced predominantly by natural killer and natural killer T-cells as part of the innate immune response, and by CD4 Th-1 and CD8 cytotoxic effector T-lymphocytes in cases of specific immunity (19). IL-4 production by leukocytes is a key regulatory event that occurs early in the type-2 immune response, which induces allergic reactions and mediates expulsion of parasites. CD4<sup>+</sup> T-cells and basophils are thought to be the key cell types that produce IL-4 during a type-2 response (20). Studies have demonstrated the reciprocal roles of IFN- $\gamma$  and IL-4 in worm expulsion. Depletion of IFN- $\gamma$  and increased expression of IL-4 with significant IgE secretion is required for worm expulsion (21). The increased IFN- $\gamma$  in *A. lumbricoides* infected children in this study is not in consonance with earlier findings (21) but the increased expression of IL-4 agrees with what was observed in a previous study (22). The raised IFN- $\gamma$  seen in our study may be attributed to a systemic inflammation in the *Ascaris lumbricoides*-infected children since the children considered for this study were apparently healthy, with no known laboratory confirmed viral, bacterial and protozoal infections. However, raised IL-4 level might be attempt by the host to expel the worm.

IL-8 is a pro-inflammatory cytokine, produced by a wide variety of cells including neutrophils, T-lymphocytes, monocytes, vascular endothelial cells, dermal fibroblasts, hepatocytes and keratinocytes. It functions majorly in neutrophil activation and recruitment (23). Th-2 lymphocytes also contribute to eosinophil differentiation and recruitment which in turn secrete IL-8 (24). Increased eosinophils are observed in acute helminth infection through helminth-induced IL-5 secretion which induces eosinophil proliferation and differentiation (25). Significantly higher serum IL-8 levels in *A. lumbricoides*-infected children compared with helminth-negative

children in this study agrees with with earlier findings (26), and may be associated with helminth-associated eosinophilia, because IL-8-mediated neutrophil proliferation and activation may occur due to *A. lumbricoides*-induced hypersensitivity reaction (27). This may also be associated with post-vaccination occurrence.

IL-6 is a highly pleiotropic molecule, with diverse pro- and anti-inflammatory properties depending on prevailing circumstance (28). It is involved in the induction of switch from neutrophil to monocytes recruitment by suppressing neutrophil-attracting chemokines and enhancing neutrophil apoptosis thereby contributing to the resolution of acute neutrophil infiltration (29). IL-6 has been reported to limit Th-2 responses, modifies Treg-cell phenotype, and promotes host susceptibility following helminth infection (30). The higher serum IL-6 levels in *A. lumbricoides*-infected children compared with helminth-negative children in our study is similar to that of Nagy et al., (31) who reported elevated IL-6 level in children with *Toxocara canis* infection. This may be attributed to the role of IL-6 as an enhancer of Th-2 cell differentiation involved in the control of helminth infection. The observed lower post vaccination serum level of IL-6 in OP-vaccinated *A. lumbricoides*-infected children compared with the pre-vaccination level might suggest inhibitory effect of *A. lumbricoides*-induced Th-2 immunity on IL-6. This may also support the function of IL-6 as promoting host susceptibility to helminth infection (30). The reduced serum levels of IL-6 and IL-8 in *Ascaris lumbricoides*-infected children after oral polio vaccination compared with serum levels before polio vaccination is an indication that decreased expression of inflammatory cytokines may be one of the mechanisms by which *A. lumbricoides* reduces efficacy of oral polio vaccine. This finding will require further investigation.

Vaccines induce immune effector cells which are majorly antibodies produced by B-lymphocytes and cytotoxic CD8<sup>+</sup> T lymphocytes that may recognise and kill evaded cells or secrete specific antiviral cytokines (32). These antibodies productions are supported by factors and signals made available by the CD4<sup>+</sup> T helper cells, which are of T helper 1 (Th-1) and T helper 2 (Th-2) subtypes (33). However, the major limitation of our study is the small sample size which compelled us to cautiously conjectured that *A. lumbricoides* infection may reduce efficacy of poliovirus vaccine because of reduction, albeit statically insignificant, in post-vaccination serum level of PV-IgA antibody in *A. lumbricoides*-infected children compared with serum PV-IgA antibody level before vaccination.

**Conclusion:**

In this study, oral poliovirus (OP) vaccination caused decrease expression of inflammatory cytokines (IL-6 and IL-8) in *A. lumbricoides*-infected school children, and *A. lumbricoides* infection may reduce PV-IgA production following OP vaccination.

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**Authors contributions:**

KSA collected the data, carried out the study and ran the data analyses. GOA conceived the study, designed the study and edited the final manuscript.

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