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Cytokine Levels and Micronutrient Status in *Ascaris lumbricoides* - Infected Nigerian School Children after Albendazole Treatment

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Abstract

Background
Intestinal helminth infection is associated with altered immune responses and deranged micronutrient status in infected children. There is no information on the status of serum cytokine levels and micronutrient status in *Ascaris lumbricoides* infected Nigerian school children at pre- and post- albendazole anti-helminthic treatment.

Aim
To provide data on serum levels of cytokines and micronutrients in *Ascaris lumbricoides* infected Nigerian children at pre- and post- albendazole anti-helminthic treatment.

Method
*T*his case-control prospective study assessed serum levels of micronutrients (zinc, iron, selenium, vitamin A, vitamin C), transferrin and cytokines (TNF–α, IFN–γ, IL-6, IL-8, IL-4 and IL-10) in 46 *Ascaris lumbricoides* infected and 40 uninfected Nigerian school children before and after anthelminthic treatment using Atomic Absorption Spectrophotometry, high performance liquid chromatography (HPLC) and Enzyme Linked Immunosorbent Assay (ELISA) as appropriate. Data were analysed using Mann-Whitney *U* test, Wilcoxon Signed Ranks Test and Kruskal Wallis Test, with levels of significance set at $\alpha_{0.05}$.

Results
More males than females were infected with helminthes and most helminth infected schoolchildren were within 5-9yrs of age. *Ascaris lumbricoides* has the highest prevalence. Most (77%) children were infected with *Ascaris lumbricoides* and 2% of the children had co-infection of Ascaris *lumbricoides* with hookworms and *Trichuris trichiuria*.
Pre-albendazole anti-helminth treatment, serum zinc and vitamin A levels were significantly lower (p=0.044 and p=0.002 respectively) while transferrin, selenium, IL-8, IL-6, IFN–γ and IL-4 levels were significantly higher (p=0.001, p=0.032, p=0.014, p=0.001, p=0.014 and p=0.010 respectively) in *Ascaris lumbricoides*-infected group compared with controls. Serum vitamin A levels were significantly higher at one month (p=0.001) and two months (p=0.001) post-albendazole treatment while IL-8 (p=0.031) was significantly lower at one month compared with pre-treatment values.

Conclusion
Inflammation, zinc and vitamin A deficiencies associated with *A. lumbricoides* infection in Nigerian children were reversed from one month of albendazole.

Recommendation
Periodic anthelminthic drug treatment coupled with taking diet containing zinc and vitamin A by school children is recommended.

Keywords: *Albendazole, Ascaris lumbricoides, Cytokines, Zinc, Vitamin A, School children.*

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Introduction

Helminth infection in humans has been dated back to pre-historic era due to incomplete eradication of the helminths. The common soil-transmitted helminths (STH) species of humans are *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm) and *Ancylostoma duodenale* or *Necator americanus* (hookworms) which infect about half a billion peopleworldwide (1).A survey estimated that as of 2010, *Ascaris lumbricoides* infected about 819 million people globally, *Trichuris trichiura* infected 464.6 million and 438.9 million people are infected with hookworm and Sub–saharan Africa accounts for 13.6%, 13.6% and 10.6% of these infections respectively (2). World Health Organisation estimates that about 846 million children worldwide are in need of drug treatment against STH infection (3).

Studies show that helminth infections are widely spread across Nigeria and about 5.7 million school children carry STH infection with *Ascaris lumbricoides* having highest prevalence followed by *Trichuris trichiura* (4, 5). Generally, helminths infection causes iron and protein loss, anaemia, nutrients malabsorption, appetite loss, reduced nutritional intake, low physical fitness, poor cognitive development and fatigue (6). Severe *Ascaris lumbricoides* infection cause abdominal distension and pain in the small intestine, lactose intolerance, as well as vitamin A and some other micronutrients malabsorption accompanied with immune derangement. This often results in diminished physical fitness, stunt growth, impaired memory and cognition with adverse consequence of reduced school attendance and impaired educational performance (7). The high burden of STH infections along with its significant negative effects are reasons for the global eradication programmes launch against parasitic infections (8).

Micronutrients play important roles in immuno-physiologic functions and vaccine efficacy (9). Helminth infection classically induces Type 2 immunity and has been linked with modulation of some Type 1 immunity – driven inflammatory diseases and down-modulation of the immune system due to helminth infection was associated with diminished responses to bystander antigens and routine vaccination (10). High prevalence especially in children, grievous economic implications, increased susceptibility to other infections and reduced vaccine efficacy are attributes of STH which necessitates the need for effective treatment of helminth infection. Study on cytokine and micronutrient levels in STH-infected Nigerian children before- and after- anthelminthic drug treatment is lacking. This is the first report which identifies the number of days after anti-helminth drug treatment when the systemic

effect of helminth infection is reversed. The outcome gives idea of when vaccine and other therapeutic management could be best administered post anti-helminthic drug treatment.

Sub–Saharan Africa still account for the greatest burden of soil transmitted helminth infection and in Nigeria, the number of school-aged children that required preventive drug treatment for soil transmitted helminth infection was estimated to be about 39 million (3). Soil transmitted helminth infections are also proposed to contribute to malnutrition and induction of chronic inflammation (7). Unfortunately, little attention is given to the possible effect of STH infection on specific micronutrients levels or on specific vaccine immune factors.

This study provided information on serum levels of cytokines, transferrin and micronutrients in Nigerian children infected with *Ascaris lumbricoides.* This was achieved by comparing serum levels of micronutrients [zinc (Zn), iron (Fe), selenium (Se), vitamin A, vitamin C], transferrin and cytokines (TNF–α, IFN–γ, IL-6, IL-8, IL-4, and IL-10) in *Ascaris lumbricoides* infected children with uninfected children before anthelminthic drug treatment, 1 month and 2 months after anthelminthic drug treatment.

Materials and Methods

Selection of Study Participants

A total of two hundred (200) school children between the ages of 5 to 19 years were recruited from Alabata and Laleye communities of Akinyele Local Government Area of Oyo State into this prospective sex/age matched case-control study. Participants were enrolled following town-hall and the Parents – Teachers Association's health awareness meetings carried out in the communities and schools respectively. Students who were aged 18 years and above gave informed consent while those aged between 15-17years gave assent in addition to the consent given by their parents/guardians. Massive deworming exercises were carried out in all the communities at the end of the study.

Participants with the following conditions were excluded from the study, viz: children whose parents gave no consent or withdrew from the study during the period of the study and children with clinical features of gastroenteritis, allergy, deformities, malaria etc.

Grouping of Ascaris lumbricoides infected participants

School children infected with only *Ascaris lumbricoides* (n = 46) were considered for micronutrient and cytokine analyses. Among *Ascaris lumbricoides* infected children, 23 schoolchildren whose parents gave consent for follow-up study were considered.

Stool Specimen Collection and Processing

Fecal specimen was scooped using spatula, put into a labeled screw cap polystyrene bottle and tightly screwed. Stool samples were collected two times from each group as follows, viz: before albendazole treatment to confirm the presence and types of helminth, at 1 month and at 2 months after albendazole treatment to confirm helminth clearance. The stool samples were temporarily stored in dark paper-rapped sample bottles placed on ice when transported to laboratory for analyses. The stool specimens were examined microscopically within 12 hours of collection using the Concentration Technique. The magnifications of X10 and X40 were used, respectively, to identify characteristic ova of the intestinal helminth.

Examination of Stool Specimen using Concentration Technique

This was performed as previously described (11). Ten milliliters of Formal/Formalin Ether Acetate Solution was added to a heap stool sample in a centrifuge tube. It was covered with plastic seal and then mixed vigorously. The mixture was centrifuged lightly at 1000g for two minutes. The supernatant was discarded, the sediment was re-suspended in normal saline and observed under X10 and X40 objective of compound microscope for diagnosis helminths.

Blood Sample Collection and Processing

Five milliliters (ml) of venous blood was obtained from each child and dispensed into plain polystyrene bottle. The blood samples were placed in upright position, allowed to retract and spun at 4000rpm for 10 minutes. The serum was removed into plain sterile cryo- precipitate tube and frozen at -20^{o}C until analysis. Blood sample collection was collected before administration of Albendazole anthelminthic drug, at one and two months after anthelminthic drug treatment.

Procedure for Anthelminthic Drug Treatment

A 400mg single dose of Albendazole tablet (Gloxosmithkline) was orally administered to each of the *Ascaris lumbricoides* infected children with the aid of water provided. Each child was observed for about 10 minutes after the administration to ensure it was not vomited.

Cytokines and Transferrin Measurement

The serum levels of IFN–γ, TNF–α, IL-4, IL-8 IL-6, IL-10 and transferrin were determined using Enzyme Linked Immunosorbent Assay (ELISA) using kits (Abcam, MA, USA, Assay Pro, MO, USA and Calbiotech, USA). The ELISA was performed as previously described

(12). All the reagents, sample and standards were allowed to attain working room temperature prior to commencement of the assay. Stock standard solution was diluted to varying concentrations to draw standard curves for the extrapolation of values. 50µl of standards and samples were added to each immunoplate well, covered and allowed to stayat room temperature for 2 hours. The immunoplate was washed 5 times using a plate washer (TECAN, Mannedorf, Switzerland).Biotinlyated Human antibody (50µl) to the cytokines and other analytes as applicable was added to each well.I t was allowed to stay at room temperature for 2 hours, after which the wash was repeated 5 times. Streptavidin – Preoxidase Conjugate (50µl) was added to each well, incubated for 30 minutes at room temperature and wash was repeated 5 times. Chromogen Substrate (50µl) was added to each well, incubated for 15 minutes inside a dark cupboard to allow the blue colour to develop. Stop Solution (50µl) was added to stop the reaction, changing the colour from blue to yellow. The absorbance of each well was read at 450nm with the aid of a microplate reader (SpectraMax 384 Plus (Molecular Devices, USA). Using a four-parameter logistic curve-fit, the unknown sample concentration was extrapolated from the standard curve.

Measurements of Trace Metals

The serum concentrations of Zinc (Zn) and Selenium (Se) were determined using Atomic Absorption Spectrophotometry (AAS) as previously described (13). Serum samples were thawed and allowed to attain room temperature and 1:20 dilution was made for each sample. Working standard solutions were prepared by diluting the stock standard with deionized water and the required standardization for each corresponding trace elements was established. Each diluted sample was aspirated into the equipment and results were displayed digitally and recorded.

Measurements of Serum Concentrations of Vitamins A and C Using High Performance Liquid Chromatography (HPLC)

Serum vitamin A and vitamin C concentrations were determined using High Performance Liquid Chromatography (HPLC) (14). A volume of 250µl of standard, controls and sample were added to 50µl internal standard, and 250µl of precipitating reagent in a 1.5ml precipitation tube. The mixture was briefly mixed using a vortex mixer and left for 30 minutes between 2°C - 8°C, then centrifuged at 10,000g for two minutes (for vitamin A) or 10 minutes (for vitamin C). Supernatant (100µl) was picked and injected into the HPLC-systemand the chromatograms are detected through the UV detector.

Statistical Analysis

Statistical data evaluation was carried out using the Statistical Package for Social Sciences (SPSS) version 21.0. Age and sex distribution of helminthes among the schoolchildren was presented as frequencies and percentages, which were analysed using 2 by 3- and 2 by 2- Chi- (X^2-) test Contingency table respectively. Serum levels of micronutrients were summarized and expressed as mean ± standard deviation and the differences two mean values were compared using the paired Student-t test. Analysis of Variance (ANOVA) was employed to compare the serum levels of micronutrients and transport proteins before and different times after anti-helminth treatment. Serum levels of cytokines were expressed as median with also the description of interquartile range. Mann-Whitney U test was used to compare differences in levels of serum cytokines between *Ascaris lumbricoides* positive group and helminth negative subjects while Kruskal Wallis Test was used to compare differences in levels of serum cytokines between *Ascaris lumbricoides* positive groups before anti-helminthic treatment, at one month of anti-helminthic treatment and at two months of anti-helminthic treatment. Level of statistical significance was set at α 0.05.

Ethics Statement

Ethical clearance was received from the University College Hospital / University of Ibadan Joint Ethics Committee and the Oyo State Ministry of Health, Ibadan, Oyo State, Nigeria.

Results

Of the two hundred (200) school children recruited, sixty [60 (30%)] were infected with helminthes (see Figure 1). More males than females were infected with helminthes while more females than males were helminth uninfected among the schoolchildren. Also, most helminth-infected schoolchildren were within 5-9yrs of age while most helminth uninfected school children were within 10-15yrs of age. There were no significant differences between ages (X^2 = 1.029, p = 0.214) and sexes (X^2 = 1.037, p = 0.309) of helminth-infected and uninfected children (Table 1). Figure 2 shows percentage distribution of helminth species in the infected children as follows: *Ascaris lumbricoides* has the highest prevalence (77%) followed by multiple infections of *Ascaris lumbricoides* and hookworms (10%) and *Trichuris trichiura (*8%).

The prevalence of school children that were not infected with helminthes was higher than those that were infected with intestinal helminthes.

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Table 1: Age and gender distribution of intestinal helminth infected school children compared with uninfected children

		Helminth Positive (%) (n = 60)	Helminth Negative (%) (n = 140)	Chi-Square	p-value
Age group	5 – 9 years	33 (32.4)	67 (27.1)	1.029	0.214
	10 – 15 years	26 (25.5)	73 (29.6)		
	16 – 19 years	1 (1.0)	0 (0)		
Gender Distribution					
	Male	33(16.5)	66 (33.0)	1.037	0.309
	Female	27 (13.5)	74(37.0)		

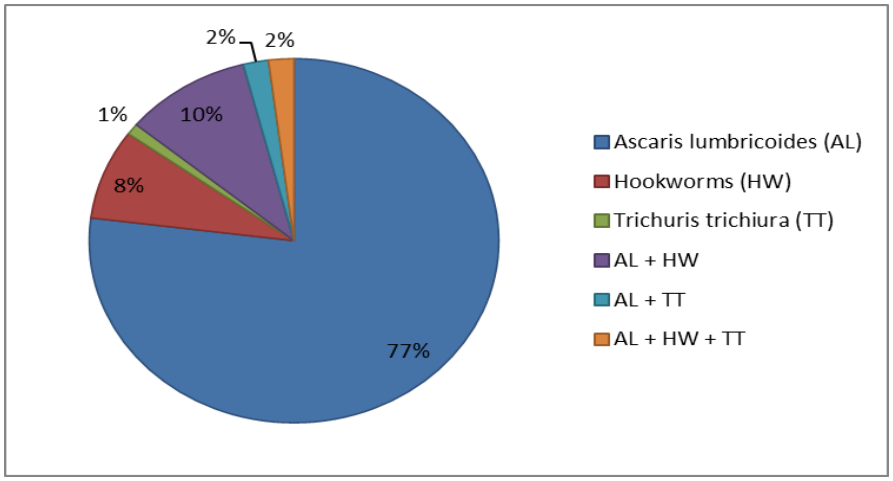

Figure 2: Distribution of intestinal helminth species among infected school children

Single infection of *Ascaris lumbricoides* (77%) was the most prevalent among infected schoolchildren, followed by hookworms (8%) while *Trichuris trichiura* (1%) was the least prevalent. There were co-infections of *Ascaris lumbricoides* with hookworms (10%), *Ascaris lumbricoides* with *Trichuris trichura* (2%) and *Ascaris lumbricoides* with both hookworms and *Trichuris trichura* (2%).

Figure 3 shows the mean serum micronutrient and transport protein levels of school children with single *Ascaris lumbricoides* infection compared with school children without helminth infection. There were significantly lower mean serum levels of zinc (139.1 ± 16.9 vs 152.7 ± 16.2 µg/dl, p=0.044) and vitamin A (119.3 ± 11.5 vs 153.6 ± 37.5 µg/dl, p=0.002) in *Ascaris lumbricoides*-infected school children compared with uninfected school aged children. Also, there was significantly higher mean serum level of selenium (62.1 ± 39.3 vs 35.5 ± 11.0 ng/ml, p=0.032) and transferrin (178.9 ± 27.5 vs 137.9 ± 28.8 mg/dl, p=0.001) in *Ascaris lumbricoides*-infected school children compared with uninfected school children. The mean

serum levels of vitamin C (2.66 ± 0.49 vs 3.05 ± 1.55 µg/dl, p=0.371), iron (170.5 ± 30.9 vs 162.4 ± 24.2 µg/dl, p=0.467), ferritin (113.6 ± 14.8 vs 106.0 ± 11.6 ng/ml, p=0.157) and haptoglobin (150.7 ± 71.9 vs 125.3 ± 16.1 µg/dl, p=0.244) were similar in *Ascaris lumbricoides*-infected school children compared with uninfected school children.

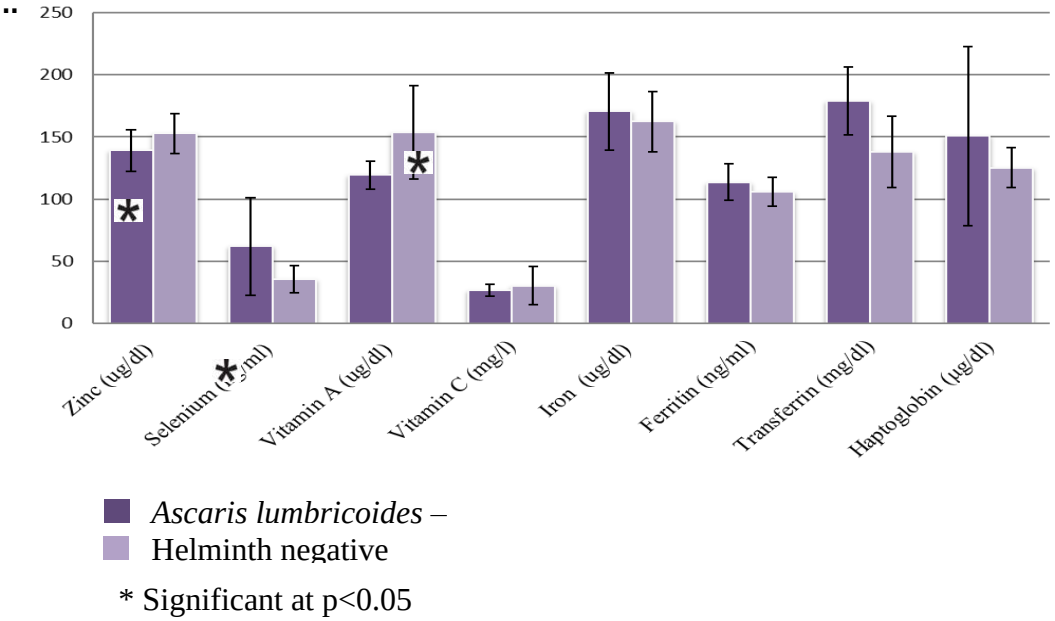

Figure 3: Serum (Mean ± S.D) micronutrient levels and transport protein of school children with *Ascaris lumbricoides* infection compared with children without helminth infection. Serum levels of Se (ng/ml) and transferrin (mg/dl) were significantly higher in *Ascaris lumbricoides* infected schoolchildren compared with uninfected children while serum levels of Zn (µg/dl) and vitamin (µg/dl) A were significantly lower in *Ascaris lumbricoides* infected schoolchildren.

Figure 4 shows the median serum cytokine levels of school children with *Ascaris lumbricoides* infection compared with school children without helminth infection. Median serum levels of IFN –γ (108.21 [IQR 75.18-137.18] vs 64.14 [IQR 25.68-88.07] pg/ml, p=0.014), interleukin-4 (204.4 [IQR 139.2-299.3] vs 89.3 [IQR 65.9-134.1] pg/ml, p=0.001), interleukin-8 (1193.9 [IQR 659.6-1321.9] vs 765.3 [IQR 218.7-802.8] pg/ml, p=0.014) and interleukin-6 (16.29 [IQR 9.92-34.79] vs 4.92 [IQR 2.69-6.52] pg/ml, p=0.001 were significantly higher in school children with *Ascaris lumbricoides* infection compared with school children without helminth infection. The median serum level of TNF–α (51.30 [IQR 40.89-61.45] vs 43.36 [IQR 36.72-52.59] pg/ml, p=0.145) and level of IL-10 (0.12 [IQR 0.07-0.52] vs 0.14 [IQR 0.13-0.36] ng/ml, p=0.724) in school children with *Ascaris lumbricoides* infection compared with schoolchildren without the infection were not statistically significant.

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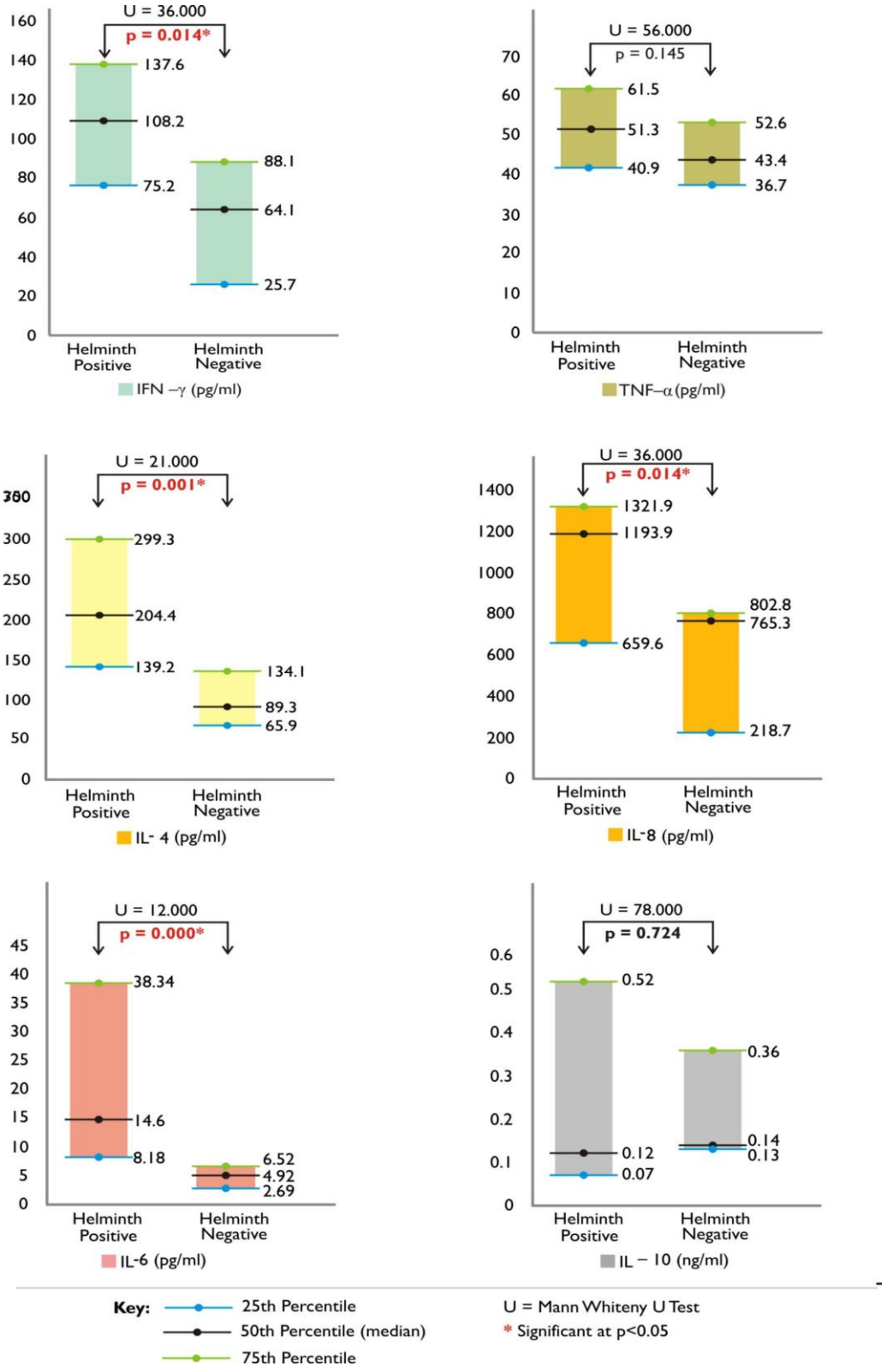

AL – Ascaris lumbricoides

Figure 4: Median serum cytokine levels of school children with *Ascaris lumbricoides* infection (n = 46) compared with children without helminth infection (n = 40). Serum levels of IFN –γ, IL-4, -6 and -8 were significantly higher in *Ascaris lumbricoides* infected schoolchildren compared with uninfected children. y axis = Cytokine concentration, x axis = Subject groups.

Table 2 compares the median serum micronutrient levels of school children with *Ascaris lumbricoides* infection before anti-helminthic drug treatment, one and two months after anti-helminthic drug treatment. Median serum vitamin A levels were significantly higher at 1 month (203.60 ± 25.90 vs 118.53 ± 9.74µg/dl, p=0.001) and 2 months' post-treatment 206.21 ± 24.11 vs 118.53 ± 9.74µg/dl, p=0.001) compared with the pre-treatment level. Median serum levels of zinc (137.93± 22.90 vs 145.04± 34.89 vs 136.73 ± 17.78, p=0.705), selenium (0.64 ± 0.11 vs 0.71 ± 0.17 vs 0.63 ± 0.41, p=0.723) and transferrin (188.01 ± 31.22 vs 197.73 ± 47.57 vs 185.85 ± 24.25, p=0.678) were not significantly different in post-treated schoolchildren compared with untreated schoolchildren.

Table 2: Serum (Mean ± S.D) micronutrient and transferrin levels in school children with *Ascaris lumbricoides* infection before and after anthelminthic treatment

	Pre-Treatment (*Al* – infected) (n=23)	1 Month Post-Treatment (n=23)	2 Months Post-Treatment (n=23)	F-value	p-value
Zinc (ug/dl)	136.73 ± 17.78	145.04± 34.89	137.93± 22.90	0.353	0.705
Selenium (ug/dl)	0.63 ± 0.41	0.71 ± 0.17	0.64 ± 0.11	0.327	0.723
Vit. A (ug/dl)	118.53 ± 9.74	203.60 ± 25.90*	206.21 ± 24.11*	66.512	0.000*
Transferrin (mg/dl)	185.85 ± 24.25	197.73 ± 47.57	188.01 ± 31.22	0.393	0.678

*** Significantly different compared with pre-treatment at p<0.05*
F = ANOVA
Al – Ascaris lumbricoides

Table 3 compares the median serum cytokine levels of school aged children with *Ascaris lumbricoides* infection before antihelminthic drug treatment, one and two months after antihelminthic treatment. Median serum levels of IL-8 were significantly lower at one-month post- anti-helminthic treatment (270.1 [IQR 167.9-566.2] vs 542.3 [IQR 320.7-935.3] pg/ml, p=0.030) but not significantly different at two months post anti-helminthic treatment (13.1

[IQR 5.8-793.9] vs 542.3 [IQR 320.7-935.3] pg/ml, p=0.158) compared with the level before anti-helminthic treatment level. Median serum levels of IL-10 and IL-6 were not significantly different at one month post anti-helminthic treatment (0.07 [IQR 0.06-0.11] ng/ml and 8.10 [IQR 6.84-11.47] pg/ml vs 0.09[IQR 0.07-0.13] ng/ml and 8.99 [IQR 7.01-12.11] pg/ml respectively, p=0.552, p=0.510) and at two months post anti-helminthic treatment (0.10 [IQR 0.07-0.18] ng/ml and 10.45 [IQR 5.68-11.66] pg/ml vs 0.09[IQR 0.07-0.13] ng/ml and 8.99 [IQR 7.01-12.11] pg/ml p=0.177 and 0.875 respectively) compared with the levels before anti-helminthic treatment. Median serum levels of IFN–γ and IL-4 were not significantly different at one month post- anthelminthic treatment (92.36 [IQR 57.18-97.04] ng/ml and 139.6 [IQR 79.6-171.9] pg/ml vs 81.59[IQR 15.64-141.39] ng/ml and 105.5 [IQR 71.9-142.9]pg/ml, p=0.875, and 0.245 respectively) and at two months post anti-helminthic treatment (76.29[IQR 51.07-125.05] ng/ml and 80.0 [IQR 32.8-159.6]pg/ml vs 81.59[IQR 15.64-141.39] ng/ml and 105.5 [IQR 71.9-142.9] pg/ml, p=0.642, and 0.778 respectively) compared with the levels before anti-helminthic treatment level. Median serum level of TNF–α was not significantly different at one and two months post anti-helminthic treatment (28.09 [IQR 22.34-41.23] vs 27.30 [IQR 23.82-33.86] vs 25.61 [IQR 21.47-30.05]pg/ml, p=0.892) compared with the level before anti-helminthic treatment.

Table 3: Median serum cytokine levels in school children with *Ascaris lumbricoides* infection before and after anthelminthic treatment

	Pre-Treatment (*Al* – infected) (n=23)	1 Month Post-Treatment (n=23)	2 Months Post-Treatment (n=23)	X^2	p-value
IFN–γ (pg/ml)	81.59 (15.64-141.39)	92.36 (57-18-97.04)	76.29 (51.07-125.05)	0.229	0.892
TNF–α(pg/ml)	25.61 (21.47-30.05)	27.30 (23.82-33.86)	28.09 (22.34-41.23)	2.382	0.304
IL–4 (pg/ml)	105.0 (71.9-142.9)	139.6 (79.6-171.9)	80.0 (32.8-159.6)	3.145	0.208
IL–10 (ng/ml)	0.09 (0.07-0.13)	0.07 (0.06-0.11)	0.10 (0.07-0.18)	2.874	0.238
IL-8 (pg/ml)	542.3 (320.7-935.3)	270.1* (167.9-566.2)	13.1 (5.8-793.9)	6.945	0.031*
IL-6 (pg/ml)	8.99 (7.01-12.11)	8.10 (6.84-11.47)	10.45 (5.68-11.66)	0.519	0.771

*Significantly different compared with pre-treatment at p<0.05 (Kruskal Wallis Test)
Al – Ascaris lumbricoides

Discussion

Intestinal helminth infection continues to be a major burden in Nigeria with children of school age living in rural areas and urban slums being most affected (15). *Ascaris lumbricoides* is the commonest helminth that infects children as reported in a previous study (16) due to close contact, playing, sleeping or eating on soil with helminth eggs.

The serum levels of zinc and Vitamin A were reduced while serum levels of selenium and transferrin were raised in infected school children compared with un-infected children before anthelminthic drug administration**.** Zinc is an integral part of many enzymes, supports antioxidant functions of selenium in glutathione peroxidase (17). Deficiency of zinc impairs functions of complement system, natural killer cells' cytotoxicity activity, neutrophils and macrophages phagocytic activity as well as generation of antioxidants by immune cells (18). There are inconsistent reports on the levels of serum Zn in helminth-infected children (12, 19, 32, 33, 34). The low levels of zinc in school children with *A. lumbricoides* infection compared with those without helminth infection as observed in this study might be associated with poor micronutrients intake, poor intestinal absorption of zinc by host due or rapid consumption zinc by helminthes (33-35) which depends on continuous supply of nutrients from the host. The implication is that low serum zinc may predispose to helminth infections or prolong helminth survival through suppression of immune responses.

Selenium is an integral part of glutathione peroxidase antioxidant enzyme and it also plays major role in regulating the expression of cytokines (20). The observed higher selenium level in *A. lumbricoides*-infected school children compared with helminth-free children before anthelminthic drug administration disagrees with earlier studies conducted in Ethiopia (19). The elevated selenium level in *A. lumbricoides* infected children is suggested to be a compensatory mechanism for low zinc level since selenium induces release of zinc by metallothioneins. In the Ethiopian study (19) elementary school children between 10 to 14 years of age (mean age 12.1±2.4) were recruited. Also, the recruited subjects in this Ethiopan study were infected with *Ascaris lumbricoides*, hookworm and *Trichuris trichura*.

This study shows insignificantly increased level of serum iron in *Ascaris lumbricoides* infected children while transferrin levels was significantly increased in the *Ascaris lumbricoides*- infected school children before anthelminthic drug administration. Raised transferrin level in *A. lumbricoides* - infected school aged children might be in response to increased level of serum iron, apart from transferrin being an antioxidant (21) which neutralizes oxidants produced during inflammatory responses which is present during

helminth infection. The implication of increased transferrin level might be an innate mechanism to reduce secondary bacterial infection in *Ascaris lumbricoides* – infected children by not making free iron available for bacterial replication (21).

The reduced serum vitamin A level in *Ascaris lumbricoides*- infected school children before anthelminthic drug administration is in consonance with previous finding (22). This might be due to low absorption and intake of vitamin A or reduced serum zinc level because zinc is important for vitamin A synthesis and release from the liver (35). Vitamin A plays major roles in immune functions as well as maintaining epithelial integrity in the respiratory and gastrointestinal tracts (23). Studies have shown that helminth – infected children absorb less vitamin A due to mucosal changes in the gastro-intestinal tract (24).

This study observed significantly increased serum level of vitamin A at one month after anti-helminthic drug treatment and further increase at two months after anti-helminthic drug treatment compared with the baseline level before anti-helminthic drug treatment. This indicates that normal absorption of vitamin A occurs within a month post-treatment of helminth infection. This supports earlier studies which reported significant rise in serum vitamin A levels following anti-helminthic treatment of infected children compared with its levels in untreated children (25) but these previous studies did not follow up the treatment to 2 months. This present study therefore suggests that anti-helminthic treatment leads to improved intestinal absorption of vitamin A in *Ascaris lumbricoides*- infected Nigerian children within one month of anti-helminthic treatment.

The elevated IL-8 levels in *Ascaris lumbricoides*- infected school children before anti-helminthic treatment compared with helminth-free children is consistent with the report of Wang *et al* (10). This might be due to eosinophilia usually associated with helminth infection. The significantly reduced IL-8 level at one month after anti-helminthic drug treatment suggests reduced proliferation of neutrophils and eosinophils within one month after anti-helminthic drug treatment. Previous studies showed that eosinophilia has been associated with parasitic diseases, particularly when the parasites invade the tissue or injure the mucosal surfaces while IL-8 which is a member of C-X-C chemokines attract eosinophils and is also synthesized by eosinophils (29, 30).

IL-6 is a highly pleiotropic molecule, which induces monocytes recruitment, suppresses neutrophil-attracting chemokines and enhancing neutrophil apoptosis (26). IL-6 has been reported to limit Th2 responses, modifies the Treg-cell phenotype, and promotes the host's susceptibility after helminth infection (27). The elevated IL-6 levels in *Ascaris lumbricoides* - infected school children before anti-helminthic drug treatment compared with helminth might be attributed to the role of IL-6 as an enhancer of Th 2 cells differentiation, involved in helminth infection control and role of IL-6 in suppressing IL-8-induced neutrophil proliferation, which occurs due to *Ascaris lumbricoides* – induced hypersensitivity reaction during re-infection (27). In this study, pre-treatment elevated serum levels of IFN-gamma, IL-4 and IL-6 were not reversed in albendazole treated schoolchildren. It is likely that residual plasma changes caused *Ascaris lumbricoides* infection persisted in circulation despite helminth clearance.

This study also reported a significantly increased serum level of IFN–γ and IL-4 in *Ascaris lumbricoides*-infected school children before anti-helminthic drug treatment compared with helminth-free school aged children. IFN-γ is a critical cytokine for adaptive and innate immunity against some bacterial, viral and protozoal infections while production of IL-4 by leukocytes is a key regulatory factor that occurs early in the Th-2 response (28), which induces allergic reactions and mediates parasites expulsion (31). Increased IFN-γ and IL-4 in *A. lumbricoides*-infected children in this study might be associated with roles that IFN-γ and IL-4 play in worm expulsion.

Conclusion

It can be concluded from this study that IFN-γ, IL-4, IL-6 and IL-8 were elevated in *Ascaris lumbricoides* infected children; and that albendazole anti-helminthic drug treatment reversed elevation of IL-8.

Study Limitation: Remoteness of participants' recruitment communities to the laboratory where the samples were processed for analyses.

Recommendation

Periodic anti-helminthic drug treatment coupled with taking fruits and vegetables containing zinc and vitamin A by school children is recommended.

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Abbreviations

TNF–α	Tumour Necrosis Factor-alpha
IFN–γ	Interferon-gamma
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-4	Interleukin 4
IL-10	Interleukin 10
AAS	Atomic Absorption Spectrophotometry
HPLC	High Performance Liquid Chromatography
ELISA	Enzyme Linked Immunosorbent Assay

Conflict Of Interest

The authors declare no financial or commercial conflict of interest.

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Authors' contribution

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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