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Anthropometric Indices and Serum Micronutrient Status of Helminth – Infected School Children from Semi-Urban Communities in Southwestern Nigeria

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Summary: Adequate nutrition is essential for normal growth of children but helminth infection is proposed to cause nutritional deficiencies. This study was carried out to assess the nutritional status of helminth infected school aged children in semi-urban communities of South-West Nigeria. Two hundred children from primary schools in Akinyele Local Government of Oyo State, Nigeria participated in the study. Anthropometric measurements were analyzed using the WHO AnthroPlus software. Kato Katz method was used to detect ova of helminths in the stool while serum levels of iron, zinc, selenium, ferritin, transferrin, vitamin A, vitamin C and haptoglobin were measured using High Performance Liquid Chromatography and Atomic Absorption Spectophotometry as appropriate. 60 (30%) of the children had intestinal helminth infection with *Ascaris lumbricoides* (23.0%) as most prevalent, followed by hookworm (2.5%) and *Trichuris trichuria* (0.5%). *S*tunting was more prevalent than thinness or underweight among the study population especially the female children. There were significantly reduced serum levels of zinc and vitamin A and significantly increased serum levels of transferrin and selenium in helminth-infected children compared with helminth-uninfected children. This study established the need for regular deworming of school age children and supplementing diets of school children in rural communities with vitamin A and zinc.

Keywords*:* Helminthiasis, Children, Nutrition, Deworming, Stunting

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INTRODUCTION

World Health Organization (WHO) reported that not less than 400 million school age children are chronically infected with soil-transmitted helminths (WHO, 2003). Out of this, about 90 million school-aged children are estimated to be infected in Africa (Brooker *et al*, 2010). The global prevalence of intestinal helminths infection in school age children was estimated to be: Roundworm 35%; Whipworm 25%; Hookworm 26 and others 14% (Partnership for Child Development, 1999). In sub-Saharan Africa, intestinal helminth infections are of major health concerns because predisposing factors such as poverty, poor sanitation, ignorance and malnutrition prevail (Crompton, 1999).

Adequate intake of micronutrients is of great importance for proper foetal and childhood development and vaccine efficacy (Failla, 2003). There is growing evidence that serum levels of multiple nutrients are reduced by helminth infection because of the inadequate intake or inefficient utilization of available micronutrients due to parasitic infestations (Rayhan and Khan, 2006). An estimated 100 million people globally, have been reported to have experienced stunting or wasting due to intestinal helminth infection (Shang *et al*, 2010; World Development Report, 2011). However, it was reported that elimination of helminth infection through deworming can reverse this trend (Stephenson *et al*, 1993).

While several studies have described the prevalence of intestinal helminth infection among school children in Nigeria (Oyewole *et al*., 2007; Awolaja and Morenikeji, 2009; Adefioye *et al*, 2011; Osazuwa *et al*., 2011), information linking intestinal helminthiasis micronutrient status is scarce. This study is aimed at establishing the relationship between intestinal helminth infection, anthropometric indices and micronutrient status among school aged children in South-West Nigeria. The result of the study may predict the need for fortification of foods in children.

MATERIALS AND METHODS

Study Site and Subjects

A total of 200 primary school children were selected from two peri–urban communities (Alabata and Laleye Communities) along Iseyin road in Akinyele Local Government Area of Oyo State, which are about 10km and 15km from the State capital (Ibadan) respectively. Each of the communities has an average population of about 500 inhabitants who are mainly petty traders and farmers. Meetings were held with the Parents-Teachers' Association to explain the aim of the study and seek their consents. Participation in the study was voluntary and participants were allowed to withdraw at any time during the study. The children were interviewed using a questionnaire to collect data on the age and sex of each child, access to drinking water, cooking method, length of stay in the village, latrine type, animal ownership, occupation and education of parents, levels of sanitation, access to healthcare and solid waste disposal system.

Ethical Consideration

Ethical approval was obtained from the University of Ibadan/University College Hospital Ibadan Research Ethics Committee and the Research Ethics Committee of the Oyo State Ministry of Health, Nigeria. Permission was obtained from the school authorities and parents before enrolling the children into the study.

Stool Samples Collection and Analysis

Stool sample were collected into a sterile universal container with scoop attached to their lids. The stools were analysed within 12 hours of collection using formol- ether concentration method for identification of helminth (WHO, 1994). Kato Katz technique was employed to quantify characteristic eggs and categorized as light, moderate and heavy intensity infections. For Ascariasis, egg per gram (EPG) of 1–4,999 EPG is light infection and ≥5,000 EPG is moderate to heavy infection. For hookworm, light-and moderate to heavy intensity infection categories are defined as 1–1,999 EPG and ≥2,000 EPG, respectively. Light infection for *T. trichiura* category was defined as 1–999 EPG and the moderate to heavy intensity infection category is defined as ≥1,000 EPG (WHO, 1994).

Blood Sample collection

About 5ml of venous blood was collected using a 23G vacutainer needle from the ante-cubital vein into a plain vacutainer bottle. Samples were allowed to clot, retract and spun at 4000rpm for 5 minutes. The serum was extracted into plain sterile cryo-precipitate tube and frozen at -20^{O}C until analysis.

Nutritional Status Assessment

The nutritional status was assessed by measuring serum levels of vitamins and trace elements.

Analysis of Micronutrients in Serum

The concentrations of micronutrients (Zn, Fe, Se) in serum were determined using atomic absorption spectrometric technique (Buck Scientific, 205, Atomic Absorption Spectrophotometer, East Norwalk, Connecticut, USA) based on the method of Kaneko (1999) as previously carried out (Arinola *et al*, 2015). This method is based on the principle that atoms of an element aspirated into AAS absorb light of the same wavelength as that emitted by the element when in the excited state. The levels of vitamins A and C were determined using High Performance Liquid Chromatography method using WATERS 616/626 (USA) machine as previously carried out (Arinola *et al*, 2015). The machine runs the sample via a suitable mobile phase and employs the interaction between the analyte and a solid phase to separate the content of the sample. Serum transferrin, ferritin and haptoglobin were assayed using commercially available Enzyme-Linked Immuno Assay (ELISA) kits (Assaypro LLC, USA) based on antigen-antibody binding as previously described (Arinola *et al*, 2014).

Anthropometric measurement

The weight, height and mid upper arm circumference of the children were measured using standard methods. The weight was taken using a digital scale which had earlier been calibrated and validated. The scales were placed on a flat surface and the children were weighed without their shoes or clothing on them. The mid-upper arm circumference (MUAC) was measured using a measuring tape. The measurement was taken at the upper arm midpoint on the left arm of the children. The weight and height were taken to the nearest units while MUAC was taken to the nearest 0.1cm.

The anthropometric measurements were analysed using the WHO AnthroPlus software to determine the nutritional status of the children as recommended by the WHO (WHO, 2005). Standard deviation (SD) scores (Z scores) was calculated to determine the weight-for-age (WAZ), Height-for-age (HAZ) and BMI-for-Age (BAZ). Overweight (> + 1SD BMI-for-age z score), obesity (> + 2SD BMI-for-age z score), thinness/ wasting (< −2SD of BMI-for-age z score), underweight (< −2SD of weight-for-age z score) and stunting (< −2SD of height-for-age (HAZ) z score) were defined according to the WHO references. Children aged 6–10 years whose MUAC was less than 13.5 cm were considered to have severe acute malnutrition (SAM) while those with MUAC between 13.5 cm and 14.5 cm were considered as having moderately acute malnutrition (MAM). For children aged 10–14 years SAM was defined by a MUAC of less than 16.0 cm while those between 16.0 cm–18.5 cm were considered as being with MAM (WHO, 2005).

Statistical Analysis

The data obtained from this study were analysed using SPSS version 17 Statistical package (SPSS Inc., Chicago, Illinois, USA). Chi square was employed to test the association between helminth status and the socio-demographic variables. Levels (mean ± SD) of the micronutrients and vitamins were compared using Student-t-test. Man-Whitney U Test was employed to compare the non parametric variables. Statistical significance was assigned for p-values less than 0.05.

RESULTS

Prevalence and intensity of helminth infection

A total of 200 school children [99(49.5%) males and 101(50.5%) females] with age range between 5 and 16 years (9.11±2.74years) participated in the study. The prevalence of intestinal helminths was 30% (n=60) with the distribution patterns of helminth infection as follows: Single infection with *Ascaris lumbricoides* (n=46, 23.0%); Hookworm (n=5, 2.5%); *Trichuris trichuria* (n=1, 0.5%); *Ascaris lumbricoides* + Hookworm (n=6, 3.0%); Ascaris *lumbricoides* + *Trichuris trichuria* (n=1, 0.5%); *Ascaris lumbricoides*+ Hookworm +*Trichuris trichuria* (n=1, 0.5%). The intensity of *Ascaris lumbricoides* infection in the children was 65.2% light infection and 34.8% moderate infection. Both Hookworm and *Trichuris trichuria* were of light intensity (Table 1). Highest prevalence of helminth infection was found among male children between ages of 5 – 9 years.

Indicators of nutritional status

The indicators of nutritional status of the children in relation to their helminth status is represented in Table 2. The mean z-scores (mean ± SD) of WAZ, HAZ and BAZ in the helminth-infected children were -0.54 ± 0.98; -0.87 ± 1.44 and -0.62 ± 1.0; and in helminth –uninfected children were -0.64 ± 1.19; -1.08 ± 1.56 and -0.64± 0.98 respectively. There were no statistical significant differences between the z-scores of WAZ, HAZ and BAZ of helminth-infected and helminth-uninfected children. The prevalence of underweight, stunting and thinness among the helminth–infected children was 6.1%, 35% and 6.7% while in helminth–uninfected children was 14.9%, 39.3% and 7.9% respectively. Highest prevalence of stunning (40%) was observed in the helminth- uninfected female children while highest prevalence of thinness (13.7%) and underweight (14.3%) was observed in the helminth –infected female children.

Micronutrient status of the children

Figure 1 shows comparison of micronutrients levels in helminth-infected and helminth-uninfected children. There were statistically significant reductions in serum levels of zinc and vitamin A in helminth-infected children compared with helminth-uninfected children. Statistically significant increases in the serum levels of transferrin and selenium were observed in helminth-infected children compared with helminth-uninfected

Table 1: Prevalence of helminth infection among the school children

	Helminth	No of cases (%)	Intensity (%)		
			Light	Moderate	Heavy
Single Infection	*Ascaris lumbricoides*	46 (23.0%)	30 (65.2%)	16 (34.8%)	
	Hookworm (HW)	5 (2.5%)	5 (100%)		
	Trichuris trichuria	1 (0.5%)	1 (100%)		
Multiple infection	*Ascaris lumbricoides* + Hookworm	6 (3.0%)			
	Ascaris lumbricoides + Trichuris trichuria	1 (0.5%)			
	Ascaris lumbricoides + Hookworm + *Trichuris trichuria*	1 (0.5%)			
Total		60 (30%)			

Table 2: Indicators of Anthropometric Status of School Children in relation to their Helminth Status

Anthropometric index (z-scores)	Helminth +ve			Helminth -ve			p-value[1]	p-value[2]	p-value[3]
	Total	Males	Females	Total	Males	Females			
Weight-for-age [mean ± SD]	0.54 ±0.98	-0.42 ±0.93	0.69 ±1.05	0.64 ±1.19	0.66 ±1.18	0.59 ±1.22	0.677	0.441	0.795
z-score<2SD (underweight) [%]	6.1	0	14.3	14.9	17.9	10.7			
Height –for-age [mean ± SD]	0.87 ±1.44	0.91 ±1.37	0.83 ±1.54	1.08 ±1.56	0.88 ±1.62	1.26 ±1.48	0.373	0.929	0.192
z-score <2SD (stunting) [%]	35	38.7	31.0	39.3	38.4	40.0			
BMI-for-age [mean ± SD]	0.62 ± 1.0	0.52 ± 0.81	0.73 ±1.18	0.64 ±0.98	0.54 ±1.04	0.73 ± 0.92	0.896	0.925	1.000
z-score<2SD (thinness) [%]	6.7	0	13.7	7.9	6.1	9.6			

p-value[1] - All helminth-infected compared with all helminth-uninfected; **p-value[2]** – Helminth-infected(males) compared with helminth-uninfected(males); **p-value[3]** - Helminth-infected(females) compared with helminth-uninfected (females).

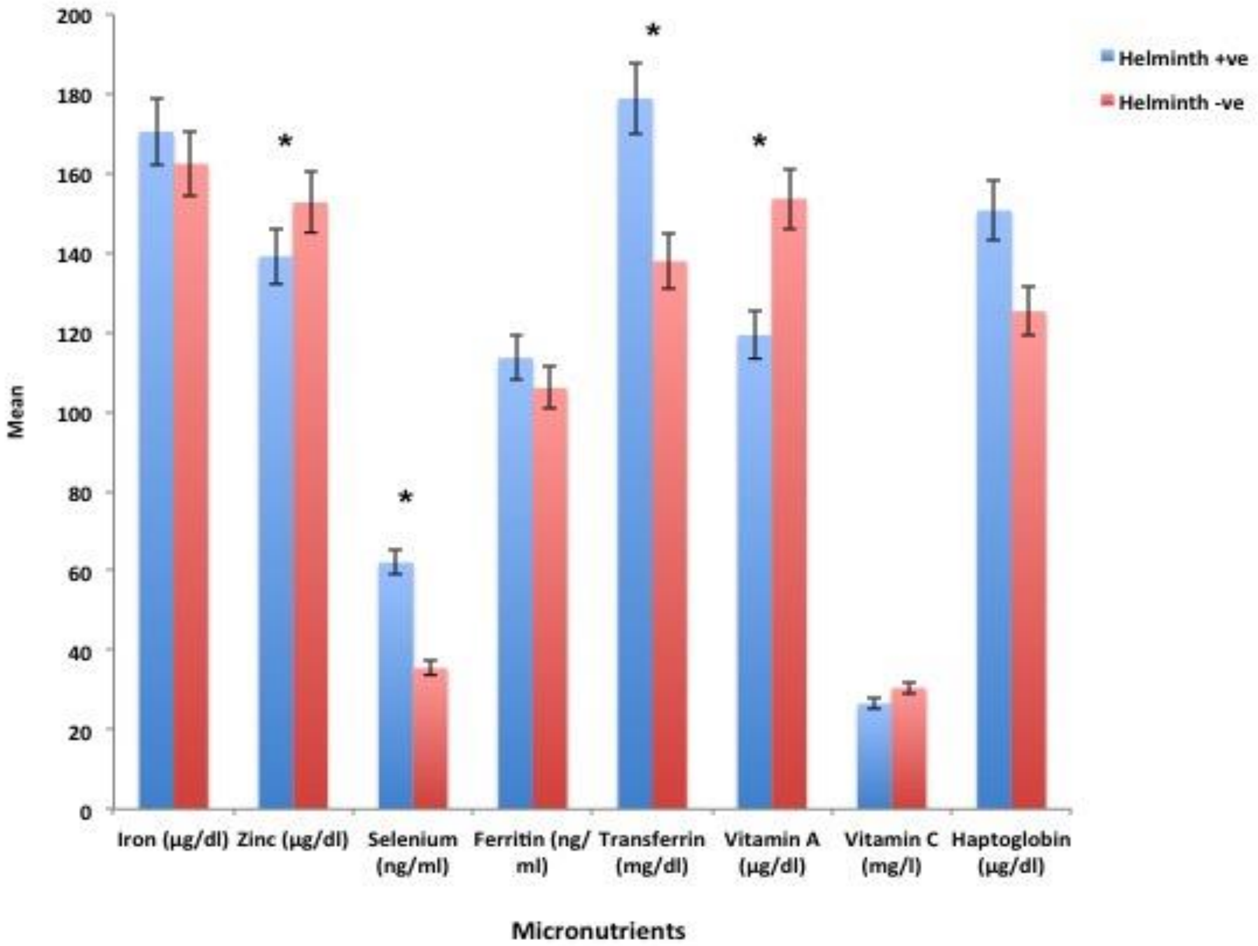

Figure 1: Comparison in the micronutrient levels between helminth-infected compared to helminth–uninfected school children

children. The increases in the levels of serum iron, ferritin and haptoglobin and reduction in serum vitamin C level in helminth-infected children compared with the helminth-uninfected children were not statistically significant.

DISCUSSION

Soil transmitted helminthiasis (STH) continues to be a major burden in Nigeria with young children of school age living in rural areas and urban slums being the most affected (Ekundayo *et al*, 2007). The present study revealed a prevalence rate of 30% helminth infection among school aged children between ages 5 – 16 years in two semi-urban areas of Ibadan in Oyo State, Nigeria. *Ascaris lumbricoides* infection had the highest prevalence of 23%, followed by hookworm (2.5%) and *Trichuris trichuria* (0.5%). The study agrees with findings of Ogunkambi and Sowemimo (2014) who reported a prevalence of 28.1% among pre-school aged children in Ile Ife, Osun State, Nigeria, but far lower than findings of Nmorsi *et al* (2009) who reported a prevalence of 94.3% in pre-school children in Ibilo, Edo State, Nigeria and Adefioye *et al* (2011) who reported prevalence of 52% in Ilie, Osun State, Nigeria.

This study also showed a high infection rate among male children and children between ages 5 – 9 years. This agrees with findings of Ogunkambi and Sowemino (2014) who reported a higher prevalence of helminths among male children and over the age of 5 years, but disagrees with findings of Adefioye *et al* (2011) who reported higher prevalence among females and in children above 15 years of age. The play patterns of male children at age 5-9 years are mostly soil-related and may be responsible for this finding,

Stunting is more prevalent in both groups of children compared with underweight and thinness. Kirwan *et al*, (2009) and Francis *et al*, (2012) reported lower prevalence of stunting and higher prevalence of underweight and thinness among children in Ile-Ife, Osun State, Nigeria and Uganda respectively. On the contrary, Amare *et al* (2012) revealed a higher prevalence of stunting and lower prevalence of underweight and thinness among school children in Ethiopia. Stunting is a type of chronic malnutrition which begins in childhood (Amare *et al*, 2012) and supplementing with quality complementary food at age 6 – 36 months minimizes negative consequences of chronic under-nutrition (Thompson *et al*, 2011). High prevalence of stunting in our study population, especially females indicates inadequate nutrition for these children after weaning which might be associated with poor nutrition education or socio-economic status, thus making them susceptible to infections.

The study showed significant reductions in serum zinc and vitamin A levels in helminth-infected compared with helminth-uninfected children and significant increases in serum transferrin and selenium levels of helminth-infected compared with helminth-uninfected children. Zinc has significant role in nucleic acid metabolism, cell replication, tissue repair, and growth (Nicola *et al*, 2002) as well as in antioxidant functions of selenium in glutathione peroxidase (McKenzie *et al*, 1998). Some studies found no association between helminth infection and zinc (Furnée *et al*, 1997; Osei *et al*, 2010) while others have reported low serum zinc in helminth infected children (Dehghani *et al*, 2011; Amare *et al*, 2012).

It has however been established that poor intake of micronutrients like vitamin A, iron, and zinc predisposes to helminth infections, prolong helminth survival and their deficiencies suppress immune responses which may conversely affect vaccine efficacy in children (Koski and Scott, 2001). Significantly reduced vitamin A and zinc levels among helminth-infected children might be due to less intestinal absorption of vitamin A due to gastrointestinal mucosal changes involving blunting of the intestinal villi and morphologic changes in the intestinal crypts following helminth infection (Tripathy *et al*, 1972). Vitamin A was suggested as useful adjuvant to vaccines (Friedman, 1991) whose deficiency leads to reduced intestinal T-helper 2 immune responses (Hurst and Else, 2012) and antibody responses to different vaccines (Ross, 2000). The implication of reduced vitamin A and zinc in helminth infected pre-school aged children is increased susceptibility to other infections and reduced vaccine efficacy.

An increased selenium level in helminth-positive children as observed in this study disagrees with earlier reports among school children in Ethiopia (Amare *et al*, 2012) and Zaire (Thorpe *et al*, 1990). Raised selenium level in the helminth infected children might be a compensatory mechanism for low zinc level. House and Welch, (1989) and Mocchegiani *et al*, (2008) reported that selenium provokes zinc release by metallothioneins via reduction of glutathione peroxidase.

This study also shows increased serum levels of transferrin, iron and ferritin in helminth-infected children compared with helminth-uninfected children. Iron functions as a constituent of haemoglobin and is also important in energy generation and functioning of the immune system (Gebre-Medhin and Birgegård, 1981). Ferritin is the major iron store in the body while transferrin is a transport protein for iron (Jason *et al*, 2001). Raised transferrin level in helminth infected children might be in response to increased level of serum iron. Iron bound to transferrin is not available for blood microbes to replicate thus raised transferrin is a likely innate mechanism to reduce secondary bacterial infection in helminth – infected children.

This study establishes heminthiasis (especially *Ascaris lumbricoides*), reduced serum vitamin A and zinc, and high prevalence rate of stunting among children of school age in the study population. Thus, the need for regular deworming of school age children and supplementing diets of school children with vitamin A and zinc.

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