

ORIGINAL RESEARCH



# Serum Micronutrients in Helminth-infected Pregnant Women and Children: Suggestions for Differential Supplementation During Anti-helminthic Treatment

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## Abstract

**BACKGROUND** The prevalence of helminth infection, which is known to affect nutritional status of the host, varies with age. The complex interplay between ages, nutrient requirements, and infection necessitated the need to recommend micronutrient supplementation during helminth infection among different age groups.

**OBJECTIVE** The aim of this study was to determine the pattern of alteration in selected micronutrients in pregnant women and preschool- and school-aged children with helminth infection.

**METHODS** We screened 245 pregnant women and 349 children for helminth infection. Of these, 17 (6.9%) pregnant women and 102 (29.2%) children (42 preschool- and 60 school-aged) had helminth infection. Only *Ascaris lumbricoides* was found in pregnant women, whereas the children had *A lumbricoides*, hookworm, *Fasciola hepatica*, and *Trichuris trichiura* infections. The helminth-infected (HI) pregnant women, preschool-aged children, and school-aged children were matched with helminth-negative (HN) pregnant women ( $n = 21$ ), preschool-aged children ( $n = 42$ ), and school-aged children ( $n = 50$ ) who served as controls. Venous blood samples were obtained and analyzed for iron (Fe), zinc (Zn), selenium (Se), and vitamins A and C. Statistical analysis was done using Student's *t* test, and  $P < 0.05$  was considered statistically significant.

**FINDINGS** Serum levels of Fe, Zn, and Se were significantly lower in HI pregnant women than HN pregnant women. In preschool-aged children, serum levels of Fe, Zn, and vitamin A were significantly lower in the HI than in the HN group. Similarly, serum levels of Zn and vitamin A were significantly lower in HI school-aged children than in the HN group. However, serum levels of Se were significantly higher in HI children (both age groups) than in the corresponding HN group.

**CONCLUSION** Helminth infection alters different types of micronutrients in children and pregnant women. Results from the present study therefore suggest monitoring Fe, Zn, or vitamin A supplementation with an anti-helminthic regimen.

**KEY WORDS** children, essential minerals, helminth infection, pregnant women, supplementation, vitamins

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## INTRODUCTION

Malnutrition and infections are common health problems in developing countries. Although the conditions can exist independently, they are intricately associated.<sup>1</sup> About 826 million people worldwide have been reported to be undernourished.<sup>2</sup> Similarly, about 2 billion people worldwide are affected by deficiency of micronutrients such as vitamins A, C, and E and essential minerals such as zinc (Zn) and iron (Fe). This undernourishment has been identified as the primary cause of immunodeficiency affecting infants, children, adolescents, pregnant women, and the elderly.<sup>1,3</sup>

Soil-transmitted helminth (geohelminth) infections are common chronic infections.<sup>4,5</sup> They are transmitted by eggs present in human feces that contaminate soil, especially in areas where sanitation is poor. The main species that infect people are the roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), and hookworms (*Necator americanus* and *Ancylostoma duodenale*).<sup>6</sup>

The 2010 Global Burden of Disease Study estimated that more than 5.2 million disability-adjusted life-years are because of helminth infection, and infection affects mainly children because of their increased behavioral risk, frequent outdoor exposure, and poor personal hygiene. Based on this information, the World Health Organization resolved that children at risk for morbidity from helminth infection are to be treated.<sup>7–9</sup>

Helminth infection is a significant burden in pregnancy.<sup>10</sup> Annually, hookworm alone infects about 44 million pregnant women.<sup>11</sup> Reports have shown that pregnant women are more susceptible to helminth infections and usually suffer from protein-energy malnutrition and deficiencies of micronutrients, such as Fe and Zn.<sup>12,13</sup> This helminth-induced chronic malnutrition increases the maternal risk for future parasitic infections and adverse pregnancy outcomes such as premature delivery, low birthweight, poor growth, and infant immune system downregulation.<sup>14–16</sup>

Diverse associations have been reported between helminth infections and micronutrient deficiencies. Poor intake of vitamin A, Fe, and Zn predisposes individuals to helminth infections, which can aggravate nutritional deficiencies, thereby helping helminth survival.<sup>17,18</sup> Helminths impair nutritional status in multiple ways. They feed on host tissues (including blood), thereby causing loss of iron and protein. They also increase malabsorption of nutrients and may compete for vitamin A in the

intestine. Furthermore, some soil-transmitted helminths cause loss of appetite, whereas some such as *T. trichiura* can cause diarrhea and dysentery.<sup>6</sup>

Existing evidence suggests that the addition of supplements to deworming programs might offer some benefits<sup>5</sup>; however, these evidences are insufficient to enable any clear or reliable suggestions to be made. It is even more difficult in developing countries (where micronutrients and helminth infections are common), as there are a limited set of clear treatment and supplementation recommendations that focus on individual micronutrients.<sup>3</sup> Additionally, there is a dearth of information on the pattern of micronutrient deficiency in vulnerable groups such as infants, children, and pregnant women who are more at risk for both malnutrition and helminth infection. The present study was carried out to determine the pattern of alteration in selected micronutrients in pregnant women and children with helminth infection.

## MATERIALS AND METHODS

**Participants.** We screened 245 pregnant women who were in their third trimester and 349 children for helminth infection. After screening, 17 (6.9%) pregnant women and 102 (29.2%) children were diagnosed with helminth infection. They were matched with 21 pregnant women and 92 helminth-negative (HN) children who served as controls. The children included 42 helminth-infected (HI) preschool-aged children (matched with 42 HN) and 60 HI school-aged children (matched with 50 HN). The pregnant women were recruited from the Adeoyo Maternity Hospital, Yemetu, Ibadan; St. Mary's Catholic Hospital, Eleta, Ibadan; and Our Lady of Apostle Catholic Hospital, Oluyoro, Ibadan, and the children were recruited from selected semi-urban areas: Gbada Alabata and Laleye communities of Ibadan.

**Informed Consent and Ethical Approval.** Participants were enrolled into the study after providing a written informed consent or assent from each of them or their parents. Ethical approvals were obtained from the Oyo State Ministry of Health and the University of Ibadan/University College Hospital Joint Ethics Committee.

**Data and Blood Sample Collection.** Demographic data were obtained using a short-structured questionnaire. About 5 mL of venous blood was obtained from each study participant and dispensed into plain bottles to obtain sera, which were stored at  $-20^{\circ}\text{C}$  until analyzed.

**Collection of Stool Specimens and Examination for Helminths.**

A fresh stool specimen was collected from each participant and placed into a labeled, leak-proof stool container (polypots) using an applicator stick. The stool specimens were examined microscopically within 12 hours of collection, using the concentration technique. Magnifications of  $\times 10$  and  $\times 40$  were used to visualize and identify intestinal geohelminth ova, respectively. The number of helminth ova was counted using Kato-Katz method.<sup>19</sup>

**Laboratory Analysis.** Serum levels of Fe, Zn, and selenium (Se) were determined using atomic absorption spectrophotometry. However, vitamins A and C were determined in the children’s serum samples only, using high-performance liquid chromatography.

**Statistical Analysis.** Differences in means of the variables were assessed using the independent Student’s *t* test. All results are presented as mean  $\pm$  SD. *P* < 0.05 was considered statistically significant.

**RESULTS**

Seventeen (6.9%) pregnant women had helminth infection. Only *A lumbricoides* infection was found in pregnant women. Of the children, 102 (29.2%) had helminth infection. Table 1 shows the distribution of helminths among the study groups. The majority of children had *A lumbricoides* infection; a few had hookworm, *Fasciola hepatica*, and *T trichiura* infections. Coinfection with different helminth species was observed in the children. Six (10%) school-aged children had *A lumbricoides* and hookworm coinfection, 1 preschool-aged child and 1 school-aged child had *A lumbricoides* and *T trichiura* coinfection, and 1 school-aged child had *A lumbricoides*, hookworm, and *T trichiura* coinfection.

As shown in Table 2, serum Fe levels were significantly lower in HI pregnant women and preschool-aged children compared with HN pregnant women or preschool-aged children. Similarly, HI pregnant women, preschool-aged children, and school-aged children had significantly lower levels of serum Zn compared with the HN groups. Furthermore, serum

levels of vitamin A were significantly lower in both groups of HI children compared with HN children. However, there was a different pattern of alteration in serum levels of Se in HI pregnant women and children compared with their HN cohorts. Se levels were significantly lower in HI pregnant women than in HN women. In contrast, serum levels of Se in the HI children were significantly higher than in the corresponding HN groups.

Serum levels of vitamin C were similar in HI children compared with corresponding HN controls (Table 2).

**DISCUSSION**

The coexistence of micronutrient deficiency and helminth infections continues to be a major health challenge affecting pregnant women and young children, especially in Sub-Saharan Africa, as a result of poor sanitation and poor personal hygiene. Although acute symptoms of helminth infections are not usually observed, studies have shown that there is a steady association between intestinal infections and reduced food intake and weight loss.<sup>3</sup>

Fe is an important micronutrient whose deficiency affects infants, children, and women of child-bearing age.<sup>20</sup> An estimated 30%–40% of preschool children and pregnant women have Fe depletion, which could cause reduced neutrophil action (with decreased myeloperoxidase activity) and impairments in cell-mediated immunity.<sup>1,21</sup> The observed lower levels of Fe in HI pregnant women compared with HN controls supports previously cited study results.<sup>22</sup> Similarly, the observed lower levels of Fe in HI preschool-aged children compared with HN cohorts supports another previous study.<sup>23</sup>

Observations from the present study could be because of blood loss, malabsorption, and poor appetite, which are characteristics of chronic helminth infection. Reports have shown that hookworm infection causes mechanical laceration and enzymatic damage to the small intestine mucosa, which could cause blood loss resulting in hypochromic microcytic anemia within 3–5 months of chronic intestinal

**Table 1. Prevalence and Types of Helminth Infection Among Pregnant Women and Children with Helminth Infection**

	AL	HW	TT	FH	AL + HW	AL + TT	AL + HW + TT
Pregnant women (%)	17 (100)	–	–	–	–	–	–
Preschool-aged children (%)	37 (88.1)	3 (7.1)	–	1 (2.4)	–	1 (2.4)	–
School-aged children (%)	46 (76.7)	5 (8.3)	1 (1.7)	–	6 (10)	1 (1.7)	1 (1.7)

AL, *Ascaris lumbricoides*; FH, *Fasciola hepatica*; HW, hookworm; TT, *Trichuris trichiura*.

**Table 2. Comparison of Serum Levels of Selected Micronutrients in Pregnant Women, Preschool- and School-aged Children With Helminth and Without Helminth Infection**

	Fe (µg/dL)	Zn (µg/dL)	Se (µg/dL)	Vitamin A (µg/dL)	Vitamin C (mg/dL)
<b>Pregnant women</b>					
HN	125.6 ± 8.4	70.8 ± 4.4	126.0 ± 7.6	ND	ND
HI	116.8 ± 11.4	66.1 ± 6.0	118.0 ± 10.3		
<i>P</i>	0.024*	0.025*	0.026*		
<b>Preschool-aged children</b>					
HN	160.3 ± 34.6	140.9 ± 22.5	39.0 ± 29.6	122.6 ± 27.2	3.04 ± 0.56
HI	111.5 ± 43.3	96.1 ± 20.0	68.9 ± 30.9	92.3 ± 21.8	2.41 ± 1.14
<i>P</i>	0.003*	0.000*	0.020*	0.004*	0.071
<b>School-aged children</b>					
HN	162.4 ± 24.2	152.7 ± 16.2	35.5 ± 11.0	153.6 ± 37.5	3.05 ± 1.55
HI	170.5 ± 30.9	139.1 ± 16.9	62.1 ± 39.3	119.3 ± 11.5	2.66 ± 0.49
<i>P</i>	0.467	0.044*	0.032*	0.002*	0.244

HI, helminth-infected; HN, helminth-negative; ND, not determined.  
\* Significant at *P* < 0.05.

infection.<sup>24</sup> Also, findings of the present study could be a result of poor absorption and the systemic effect of infection and utilization of Fe by microorganisms for their growth and multiplication.<sup>25</sup>

One previous study reported that helminthes such as *A lumbricoides* could impair Fe absorption in the duodenum and jejunum.<sup>26</sup> Because anemia is a common observation in pregnancy (due to blood volume expansion, frequent vomiting, and poor appetite), observations from the present study indicate that helminth infection in pregnancy could aggravate pregnancy-associated Fe deficiency. This suggests that screening of pregnant women in helminth-endemic areas for possible infection could help to identify pregnant women who might benefit from Fe supplementation to prevent possible helminth-attributable Fe deficiency in pregnancy.

Although the preschool-aged children were not offspring of the mothers studied, there is a possibility that their mothers are Fe deficient, as the majority of the children were still being breastfed. This observation could be supported by insignificant differences in Fe levels in HI school-aged children (compared with HN children) who possibly could be consuming diets that are adequate in Fe unlike the preschool-aged children who solely depended on the breast milk (which may be Fe deficient) of their mothers.

Zinc is an essential mineral required for the activities of more than 300 enzymes involved in carbohydrate and protein metabolism, heme biosynthesis, and carbohydrate transport. It plays an important role in immune response because its deficiency reduces nonspecific immunity (neutrophil and natural killer cell and complement activity),

reduces numbers of T and B lymphocytes, and suppresses delayed hypersensitivity, cytotoxic activity, and antibody production.<sup>1</sup> The observed lower levels of Zn in HI preschool- and school-aged children compared with HN children are in line with those previously reported.<sup>27,28</sup> Similarly, the observed lower Zn levels in HI pregnant women compared with HN women is not surprising. It has been reported that Nigerian pregnant women experience Zn deficiency.<sup>29</sup> The lower Zn levels observed in the present study in all HI groups (compared with HN groups) could be because of low dietary intake (due to loss of appetite), gastrointestinal bleeding, malabsorption, diarrhea, or infection. Zn deficiency decreases resistance to infectious diseases by interfering with the ability of T cells to produce interleukin-4, an important cytokine required to drive an optimum T-helper 2 response, thereby causing defective immunoglobulin (Ig)E response, which is vital in control of helminth infection.<sup>30,31</sup> Children with malnutrition and helminth infection coexistence usually have high concentration of total IgE, which are not protective as they are not worm-specific and their memory T cells do not recognize helminth antigens.<sup>31,32</sup> These reports probably explain why certain parasites survive better in a Zn-deficient host than in a well-nourished host.

Se is an important micronutrient for effective immune response. It is an integral component of glutathione peroxidase, selenoprotein-P, and thioredoxin reductase.<sup>33</sup> Dietary intake of Se varies as its concentrations in plant-based foods reflect the concentrations in the soil in which the plants were grown. Similarly, Se concentrations in animal

sources of food depend on the Se content of the plants used for forage or whether animal feed was fortified with Se.<sup>33</sup> There is growing evidence that serum levels of Se and some other essential micronutrients are reduced by helminth infection.<sup>5</sup> The lower levels of Se seen in HI women in our study compared with HN women could suggest increasing gastrointestinal loss through bleeding, malabsorption, and poor appetite, which are associated with chronic helminth infection. Although Se deficiency has been reported in pregnant Nigerian women,<sup>34</sup> its further reduction in HI women (as well as reduction in Fe and Zn as observed in the present study) could be induced by helminth infections by diverting the micronutrients toward immune response with a view to eliminating the helminths. Therefore, there is a need to identify HI pregnant women with a view to initiating appropriate clinical intervention to reduce possible maternal and fetal morbidity and mortality attributable to essential micronutrient deficiencies. This is very important in Nigeria, as most rural Nigerian women, including those who are pregnant, consume cereal- or legume-based diets and have little access to animal products or a variety of fruits and vegetables, which are rich sources of essential micronutrients.<sup>35</sup>

Coinfection by multiple helminth species is common, and infection by a single species is usually a risk factor for becoming infected with another species.<sup>36</sup> The higher levels of Se in HI preschool- and school-aged children in the present study, compared with HN children, cannot be presently explained. Helminth infections often are studied together, but alteration in serum micronutrients might be species dependent because of their distinct lifecycles and pathogenesis.<sup>18</sup> Although *A lumbricoides* infection was the most prevalent among the children, infection with other helminths, such as hookworm and *T trichiura*, as well as the observed coinfection with different helminth species might be responsible for our observation. Therefore, species-specific studies are needed to shed more light on our observation.

Vitamin A is a fat-soluble vitamin that maintains the integrity of epithelium in the respiratory and

gastrointestinal tracts.<sup>1</sup> Vitamin A deficiency increases susceptibility to infections and is a cause of 1.2 to 3 million child deaths per year.<sup>37</sup> Experimental studies have shown that vitamin A deficiency reduces intestinal T-helper 2 immune response against nematode infections.<sup>38</sup> Although the exact mechanism through which helminth infections cause vitamin A deficiency remains unclear, the relationship between vitamin A deficiency and helminth infections has potentially important consequences for health, especially in areas where malnutrition, poor sanitation, and hygiene are common.<sup>5</sup> The observed low vitamin A levels in both groups of HI children compared with HN children agree with findings from a previous study.<sup>39</sup> Findings from the present study could be caused by vitamin A malabsorption in HI children as a result of gastrointestinal mucosal changes involving blunting of the intestinal villi and morphologic changes in the intestinal crypts following helminth infection.<sup>40</sup> Another study reported that HI children absorb less vitamin A after supplementation.<sup>39</sup> Additionally, results from the present study could be due to helminth-induced impaired intestinal fat absorption, which results in reduced vitamin A absorption. In preschool-aged children, the observed low vitamin A could be due to Zn deficiency. Inadequate Zn supply has been shown to prevent normal release of vitamin A from the liver.<sup>1</sup>

## CONCLUSION

Based on the observations from the present study, it is clear that helminth infection alters levels of different micronutrients in children compared with pregnant women. Therefore, HI children and pregnant women might benefit from individualized dietary intervention (such as biofortification) and possible supplementation with anti-helminthic treatment. There is also a need for public enlightenment on dietary sources and the right quantities of essential micronutrients to be consumed to ameliorate alteration caused by helminth infections commonly found in poor hygienic and malnourished settings.

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