A significant association between intestinal helminth infection and anaemia burden in children in rural communities of Edo state, Nigeria

Favour Osazuwa, BMLS., AIMLS., AIMLTA.,1 Oguntade Michael Ayo, PhD.,2 Paul Imade, MSC., FIMLS.1

1Department of Medical Microbiology, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria.
2Department of Biomedical Laboratory Sciences, Ladoke Akintola University of Technology, Ogbomosho, Nigeria.

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Abstract

Background: Anaemia is estimated to affect half the school-age children and adolescents in developing countries. Aim: This study aimed to determine the prevalence of anaemia and evaluate the relationship of intestinal helminth infection on the anaemia status of children in the rural communities of Evbuomore, Isiohor, and Ekosodin, in the Ovia North East local government area of Edo State, Nigeria. Subjects and Methods: Faecal samples and blood samples were obtained from 316 children aged 1-15 years. Faecal samples were examined using standard parasitological techniques, and anaemia was defined as blood haemoglobin <11 g/dL. Results: Of the 316 children, 38.6% were anaemic: 75.9% of children in Evbuomore, 42.3% in Isiohor and 26.8% in Ekosodin. The overall parasite prevalence in the three communities were: Ascaris lumbricoides (75.6%), hookworm (16.19%) and Trichuris trichiura (7.3%). Malnutrition was patent; 37.0% of the children were stunted, 19.3% wasted, and 44.0% underweight. There was a statistically significant association between hookworm and Ascaris lumbricoides infection and anaemia (P <.001). Serum ferritin levels were more sensitive than haemoglobin in detecting anemia and were correlated with intestinal helminth infection. Conclusion: Intestinal helminth infection in a concomitant state of malnutrition is observed in this population. Intervention programmes should be aimed at control of intestinal helminth infection and iron supplementation.

Keywords: Anaemia, Children, Iron deficiency anaemia, Intestinal helminth infection.

Introduction

Anaemia continues to be a major public health problem worldwide. According to estimates of the World Health Organization, two billion people suffer from anaemia in the world [1]. The highest prevalence of anaemia exists in the developing world where its causes are multifactorial, ranging from micronutrient deficiencies, including iron, folate, and vitamin B12; and infectious diseases [2]. Iron deficiency anaemia is thought to affect the health of more than one billion people worldwide, and it is the most common form of anaemia in the developing world [3]. Iron deficiency anaemia accounts for most of the anaemia that occurs due to parasitic infections. Most seriously affected are young children and women in less developed countries [4]. The susceptibility of children to parasitic infections is due to their lower immune response compared to adults, poor hygiene, and poor sanitary and environmental conditions which favour the development of parasites and eventual infection of hosts [3]. Anaemia in children has been related to reduced work performance, reduced cognitive functions, growth retardation, and impaired immune systems [5].

Anaemia is often associated with parasitic disease such as malaria and hookworm infections [6, 7]. Hookworms contribute to anaemia because it induces iron deficiency
by chronic intestinal blood loss. The two species of hookworms *Ancylostoma duodenale* and *Necator americanus* cause about 0.2 mL and 0.15 mL blood loss per day respectively. Hookworms also release anti clotting factors (i.e., coagulase, a blood thinner) which ensures continuous blood flow [7]. High intensity *Trichuris* and *Ascaris* infections have been known to influence nutritional status [7].

In an earlier report [8], a non-significant association between malaria parasitaemia and anaemia was reported. This work reports on the prevalence of anaemia in the rural communities of Evbuomore, Isiohor and Ekosodin of Edo state, Nigeria, and evaluates the association between intestinal helminthiasis and anaemia status.

**Subjects and Methods**

Prior to the commencement of the study, permission was obtained from the Local Education Authority of the Ovia Local Government Area, and from the elders of the communities. The study was carried out in Isiohor, Evbuomore and Ekosodin villages located in Ovia Local Government area of Edo State, Nigeria between February and June, 2010. The villages are good representations of the area in style of living, culture and occupations practiced.

Both male and female children aged 1-15 years in schools and healthcare centres made up the study population. Personal hygiene and environmental sanitation information were obtained about the children; this was done directly through the teachers or health centre nurses who were familiar with the local conditions. Information on age, sex, and nutritional levels were obtained with a questionnaire. Anthropometric measurements included height and weight using a standing scale and a paper stadiometer attached to a straight wall.

**Sample collection**

Labeled wide-mouthed specimen bottles with screw caps were distributed to participating school children a day before the study. The children were instructed to bring freshly passed stool the following day. Instructions on how to avoid contamination were also given to each child, and the time of stool collection noted. Only stool samples collected within 2 hours of delivery were used in the study. A blood sample of 5mL of venous blood was carefully drawn into potassium EDTA-containing tubes.

**Sample analysis**

Laboratory analysis of the samples was done at the Medical Microbiology Laboratory of University of Benin Teaching Hospital, Benin City, Nigeria.

The faecal samples were examined for parasites using the method described by the World Health Organization [9]. Microscopic examination of a stool sample was followed by direct saline and iodine preparation. A portion of the stool sample was emulsified in normal saline on a glass slide, covered with a cover slip, and examined microscopically using 10X and 40X objectives for ova of parasites. A drop of iodine solution was added to a homogenous saline preparation of the stool sample, covered with a cover slip, and examined microscopically using 10X and 40X objectives for the cysts of parasites.

Samples that were negative for parasite ova and cysts were subjected to a concentration method as described by the World Health Organization [9]. The formal ether concentration technique was used. A 1g sample of the stool was emulsified in 5 mL of formal saline, sieved, and the suspension collected in a centrifuge tube, followed by the addition of 3mL of ether. The well-mixed suspension was centrifuged at 3000 rpm for 1 minute. The supernatant was discarded, and the sediment examined microscopically using 10X and 40X objectives for cysts and ova of parasites.

**Haemoglobin concentration estimation**

Haemoglobin concentration (Hb) was determined by photometry, using a Sysmex KX-21N haemoglobin auto-analyzer instrument [10]. Anaemia was defined as a haemoglobin concentration <11g/dL.

An enzyme immunoassay kit for the determination of ferritin in serum (BIOTEC Laboratories Ltd, UK) was used to test 122 blood samples from the three villages following the manufacturer’s directions. Briefly, the assay detects serum ferritin using two specific monoclonal antibodies directed against two different epitopes on the serum ferritin molecule. The capturing monoclonal antibody is conjugated to biotin, while the second monoclonal antibody is labeled with horseradish peroxidase, which reacts with the chromogen, tetramethylbenzidine (TMB). Human liver ferritin standards containing 0, 5, 20, 100, 400, and 1000 ng/mL ferritin were used to produce a standard curve. Plates were read at an absorbance of 405 nm. Absorbance values were converted to concentration using the ferritin standard curve.

**Statistical analysis**

A correlation analysis to determine the relationship between anemia and physical status (wasting, stunting, and underweight) was carried out using SPSS software. The dependent variables Hb and mid-upper arm circumference (MUAC) were regressed on the dummy variables of normal, mild- moderate and severe malnutrition as arrived at using the categories of weight for height (W/H), height for age (H/A) and weight for age (W/A). The models allowed us to know if there was any significant difference between the Hb and MUAC of normal and malnourished children. Two dummy variables were introduced into the regression model to avoid perfect co-linearity of regressors. The category of normal nutritional status was used as the base dummy, in order to allow the directly estimated coefficients of the dummy variables in the model to be estimated as the deviation in the level of the dependent variable of the normal from that of the category in question.
Results
A total of 316 school children were enrolled in the study: 142 from Isiohor, 32 from Evbuomore, and 144 from Ekosodin. There were 162 (51.26%) boys and 154 (48.73%) girls. The mean age of the children was 5.67 years. The overall prevalence of anaemia in the three communities is shown in Table 1.

Table 1 Prevalence of anaemia in three communities

| Community | Anaemic (Hb<11g/dL) | Non-anaemic (Hb>11g/dL) |
|-----------|---------------------|-------------------------|
| Isiohor   | 60 (42.3)           | 82 (57.7)               |
| Evbuomore | 24 (75.0)           | 8 (25.0)                |
| Ekosodin  | 38 (26.8)           | 104 (73.2)              |

Table 2 Prevalence of parasites by age and sex of children

| Parasite          | Male | Female | Age, years |
|-------------------|------|--------|------------|
| Hookworm          | 24 (21.8) | 12 (10.7) | 1-5 6-10 11-15 |
| Ascaris lumbricoides | 76 (69.1) | 92 (82.0) | 6 (76.5) 46 (79.3) 20 (66.7) |
| Trichuris trichiura | 10 (9.0) | 8 (7.1) | 4 (2.9) 6 (10.3) 8 (26.7) |
| Total             | 110 (49.5) | 112 (50.5) | 136 (60.7) 58 (25.9) 30 (13.4) |

Table 3 Types and degree of malnutrition

| Type of malnutrition | Number (%) |
|----------------------|------------|
| Stunting             | 117 (37.0) |
| Wasting              | 61 (19.3)  |
| Underweight          | 139 (44.0) |

Table 4 Association of intestinal helminth infection on anaemia burden

| Parasite          | Anaemic (Hb<11g/dL) | Non-anaemic (Hb>11g/dL) | P value |
|-------------------|---------------------|-------------------------|---------|
| Hookworm          | 24 (66.7)           | 12 (33.3)               | <.001   |
| Ascaris lumbricoides | 40 (47.1)          | 45 (52.9)               | .01     |
| Trichuris trichiura | 4 (22.2)           | 14 (77.8)               | .30     |

Table 2 summarizes the overall sex- and age-related prevalence of parasitaemia. Parasitaemia prevalence was similar in both sexes. The age group from 1-5 years had the highest parasite prevalence, followed by the age group 6-10 years and 11-15 years (P < .001). A. lumbricoides showed the highest number of cases in all age groups.

The nutritional status of the study children was based on underweight and stunting as defined in the Methods. The total number and percent of children judged to be malnourished was shown. The type and degree of malnutrition in the study children is shown in Table 3. Underweight was the most prevalent form of malnutrition, while the most severe form was stunting. There was a significant positive correlation between stunting and underweight (P < .001). Regression analysis showed that mid-upper arm circumference (MUAC) differed significantly between children with a normal nutritional status and those severely malnourished (P = .01). Serum ferritin levels were more sensitive than haemoglobin concentration in detecting anemic children. About 9.8% (12/122) had serum ferritin values near normal (i.e., less than 10 ng/mL). Serum ferritin concentration was significantly lower among children in Evbuomore (10 ng/mL) than among children in Isiohor and Ekosodin (175 ng/mL) (P < .001). Serum ferritin concentration correlated with intestinal helminthiasis, so anaemia in this population is probably associated to a concomitant intestinal helminthiasis within a superimposed deficiency in iron.

Table 4 shows the relationship between individual parasite types and anaemia. Hookworm and Ascaris lumbricoides infections were independent risk factors for anaemia (P < .001).

Discussion
Anaemia is very common in developing countries and a multi-country survey in sub-Saharan Africa showed that it is generally a serious problem in school children [11]. In the rural areas of Edo state, Nigeria, anaemia in children was shown to be a worrying public health problem in a very recent report of a study we carried out [8], and a prevalence of 38.6% for anaemia was recorded. The mean age of children in this study was 5.67 years, which is within the age range that is at the highest risk of anaemia as reported by Calis et al [5]. When compared to the two other communities studied, Evbuomore had a higher prevalence of anaemia. In this area, the mean age of the children studied was 2.75 years, which may explain a marked reduction in haemoglobin concentration, as most of the children at this age are in rapid growth spurts.

This study showed that although Ascaris lumbricoides was the most prevalent parasite in both sexes, none of the parasites were gender-dependent, in conformity with a previous report [12]. Ascariasis decreased gradually in prevalence as the age of the children increased. However, the high prevalence of parasitaemia in virtually all age groups indicates general ignorance and/or deliberate neglect of basic rules of hygiene among the children. The observation of a relatively higher prevalence of hookworm in the age group 1-5 years may be an indication of poor hygienic practices. However, the prevalence of T. trichiura is similar in all age groups, and this can be explained by frequent contact with infected stool when children play outside.

Hookworm infection is causally linked to anaemia in
Moderate or high intensity Trichuris infections are also associated with anaemia [18]. The prevalence of 7.3% recorded in this study agrees with that of Agbolade et al [13] and Nmorsi et al [14]. Considering T. trichiura in this study as an independent risk factor for anaemia, there was no significant association between hookworm and anaemia status. This report agrees with previous studies of Stoltzfus et al [16], but disagrees with that of Hung et al [17] where no association was established between hookworm infection and anaemia among children of ethnic minority of Phan tien village in Vietnam.

The prevalence of 75.6% in this study for Ascaris is in congruence with most recent reports [13, 14], where a high prevalence of ascariasis among the subjects studied was corroborated by the relatively high occurrence of unhygienic habits among them. Ascariasis is known to influence nutritional status [20], but its impacts on anaemia is less clear. There was a significant association between ascariasis and anaemia. Other studies have used height, weight or haemoglobin concentrations as indices to assess the nutritional status of parasite-infected children and children treated for parasites, and show a remarkable improvement in haemoglobin status in children given iron supplementation, thus reiterating the contribution of worm infestation to childhood malnutrition [21].

Serum ferritin levels as measured by ELISA can be reliably used to diagnose anaemia, as this study has shown. This test for anaemia is very sensitive and easily done. There was a significant association between serum ferritin values and intestinal helmint infection in this study; this suggests that the anaemia measured in this population may have been due to a concomitant intestinal helminth infection and malnutrition.

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