Contribution of malnutrition and malaria to anemia in children in rural communities of Edo state, Nigeria

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Abstract
Background: The most common cause of anemia is an iron deficiency; however, the condition may also be caused by deficiencies in folate, vitamin B₁₂ and protein. Some anemia is not caused by nutritional factors, but by congenital factors and parasitic diseases such as malaria. Aim: This study attempted to estimate the prevalence of anemia among children in three rural communities of the Ovia North East Local government area, and to determine whether its cause was nutritional or could be attributed to malaria. Patients and Methods: A total of 316 children between the ages of 1 and 15 years were included in the study. Children were examined for malaria parasites by microscopy. The World Health Organization (WHO) age-adjusted cut-off for hemoglobin was used to classify anemia. Results: 38.6% of the children were anemic, with hemoglobin levels lower than 11g/dL, although parasite prevalence and density were low. Malnutrition was patent; 37.0% of the children were stunted, 19.3% wasted and 44.0% underweight. Serum ferritin was more sensitive than hemoglobin concentration in detecting anemic children. Anemia was also significantly higher in the Evbuomore village school than in the Ekosodin and Isiohor villages (P<0.001). Conclusion: Anemia detected in this population may be due more to malnutrition than to malaria.

Keywords: Anemia, malnutrition, children, iron deficiency, malaria.

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Introduction
Anemia continues to be a major public health problem worldwide. According to estimates from the world Health Organization, two billion individuals suffer from anemia in the world [1].The highest prevalence of anemia exists in the developing world where its causes are multifactorial, ranging from micronutrient deficiencies such as iron, folate, vitamin B₁₂ to infectious diseases such as malaria and worm infections [2]. Iron deficiency anemia is thought to affect the health of more than one billion worldwide and it is the most common form of anemia in the developing world [3].

Iron deficiency anemia accounts for most of the anemia cases that occur due to parasitic infections. Most seriously affected are young children and women in less developed countries [4]. Iron requirement varies from one stage in life to the other. It is very high in pre-term babies, low birth weight infants, adolescents, and in pregnancy, when an extra supply of iron may be necessary [5]. Recent research indicates that iron deficiency has important implications, which include less learning ability and behavioral abnormalities in children, a lower ability to work hard, and poor appetite and growth[3].

The aim of this study was to estimate the prevalence of anemia in three rural villages in the Ovia Local Government area of Edo State, Nigeria and to determine whether its cause was nutritional or due to malaria infection.
Patients and Methods
The study was performed in the Isiohor, Evbuomore and Ekosodin villages located in the Ovia Local Government area of Edo State, Nigeria. The villages were selected for the study, as the population was well homogenized without much difference in style of living, culture and occupations practiced.

Both male and female children aged 1-15 years from schools and healthcare centers comprised the study population. Prior to the commencement of the study, permission was obtained from the Local education authority of Ovia Local Government Areas and from the elders of the community. Personal hygiene and environmental sanitation information were provided by the children as well as through the teachers or health center nurses who were familiar with the local conditions. Information on age, sex and nutritional level were obtained with an administered questionnaire.

Blood samples were collected by antecubital venipuncture by qualified personnel from the Ekosodin Health Center into sample tubes containing anticoagulant (citric acetate) and stored on ice for transport to the laboratory later in the day. The weight, height and mid upper arm circumference (MUAC) measurements were taken following standard procedures and necessary precautions. Laboratory analysis of the samples was conducted at the medical microbiology laboratory of the Department of Medical Microbiology, University of Benin Teaching Hospital, Benin City, Nigeria.

Malaria was investigated during the study by microscopic examination of Giemsa stained thick blood smears [6]. Malaria parasite densities were calculated as used elsewhere [7]. Malaria parasites were counted against 100 white blood cells assuming a reference value for African white blood cells to be 6,000 /cm3. Hemoglobin concentration was measured using Sysmex KX-21N hemoglobin auto analyzer instrument (Sysmex Corporation, STATE) [8]. Anemia was defined as Hb<10g/dL [7].

An enzyme immunoassay kit (BIOTEC Laboratories Ltd, UK) for the determination of ferritin in serum was used to test 122 samples from the three villages. The assay was based on the principle of a sandwich formed by the serum ferritin, which is detected between two specific monoclonal antibodies directed against two different epitopes on the serum ferritin molecule. The capturing monoclonal antibody was conjugated to biotin, while the second monoclonal antibody was labeled with horseradish peroxidase. The plates had been pre-coated with streptavidin. 20u1 of serum and ferritin standards (containing different concentrations of human liver ferritin: 0, 5, 20, 100, 400, 1000ng/ml in human protein buffer with gentamycin) were added to all but the blank wells in row A. 100u1 of a preparation containing the two monoclonal antibodies to ferritin were also added and the whole incubated at room temperature for 1 hour. The plates were then washed five times with phosphate buffered saline with Tween 20. The chromogen, tetramethylbenzidine (TMB) was mixed in equal volume with the substrate solution containing stabilized hydrogen peroxide and 200u1 of chromogen-substrate mixture was dispensed into the wells. After incubation at room temperature (29°C) for 10 minutes in the dark, 100u1 of IN hydrochloric acid was added to stop the color reaction. Plates were read at an absorbance of 405nm. Absorbance values were converted to their concentration by reading from the ferritin standard (dose response) curve.

Statistical analysis
A correlation analysis to determine the relationship between anemia and wasting, stunting and underweight was performed using the SPSS software. Each of the dependent variables Hb and MUAC was regressed on the dummy variables of normal, mild-moderate and severe malnutrition as determined by using the categories of weight for height (W/H), height for age (H/A) and weight for age (W/A). The models enabled us to determine 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; 140 from Isiohor, 32 from Evbuomore and 144 from Ekosodin. 162 (51.26%) were males and 154 (48.73%) were females. The mean age of the children was 5.67 years. The overall prevalence of anemia in the three communities was 38.6%, 75.0%, 42.3% and 26.8% for Evbuomore, Isiohor, and Ekosodin, respectively (P<0.001) (Table 1).

| Community     | Anemic(Hb<10g/dL) | Non-Anemic(Hb>10g/dL) |
|---------------|-------------------|-----------------------|
| No. (%)       | No. (%)           |                       |
| Isiohor       | 60(42.3)          | 82(57.7)              |
| Evbuomore     | 24(75.0)          | 8(25.0)               |
| Ekosodin      | 38(26.8)          | 104(73.2)             |
| P<0.001       |                   |                       |

Table 2 Prevalence of parasites in relation to gender and age of children

| Parameters | No. Examined | No. Infected (%) |
|------------|--------------|------------------|
| Sex        |              |                  |
| Male       | 162          | 58(35.8)         |
| Female     | 154          | 48(31.6)         |
| Total      | 316          | 106              |
| Age        |              |                  |
| 1-5        | 72           | 53(73.6)         |
| 6-10       | 165          | 32(19.4)         |
| 11-15      | 79           | 21(26.6)         |
| Total      | 316          | 106              |
The pooled prevalence of malaria parasitaemia was 33.5%. Table 2 shows the age- and gender-related prevalence of parasites. The age group of 1-5 years had the highest parasite prevalence followed by 6-10 years and 11-15 years. Table 3 shows the prevalence of malaria parasitaemia in the three communities studied, with the highest prevalence of parasites in the Ekosodin community. Table 4 demonstrates the relationship between malaria parasitaemia and anemia (p=0.06).

Subjects were used to determine the nutritional status based on underweight and stunting (i.e. W/Age and H/A, respectively)(Table 5). 36% of the children were stunted, 18.3% wasted and 44.2% underweight. 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 <0.001). Regression analysis showed that mid-upper arm circumference (MUAC) differed significantly between children with a normal nutritional status and those severely malnourished (P = 0.01).

The hemoglobin values were significantly different between normal and mild to moderate malnourished children. Mean parasite density for those with malaria parasitaemia in the three villages were similar, but a marked heterogeneity in indices was found in the three villages studied.

The serum ferritin levels were more sensitive than hemoglobin in detecting anemic children. About 9.8% (12/122) had serum ferritin values near normal (i.e., less than 10 μg/mL). Serum ferritin concentration was significantly lower among children in the Evbuomeore (110 ng/ml) than among children in Isiohor and Ekosodin (175 ng/ml) (P <0.001).

Serum ferritin concentration did not correlate with the presence of malaria parasites, therefore, anemia in this population was likely not due to current Plasmodium falciparum infections. There was no significant difference in serum ferritin values in age groups 1-5 years (150 ng/ml), 6-10 years (153 ng/ml) and 11-15 years (115 ng/ml); all age groups were anemic.

**Discussion**

Anemia is common in developing countries and a multi-country survey in sub-Saharan Africa showed that it is generally a serious problem in school children [9]. This study recorded a prevalence of 38.6% for anemia, which confirmed that anemia in school children remains a public health concern in Edo State. It has been reported that preschool children (<8years) and adolescents (>15 years) during growth spurts have the greatest physiological demands for iron and are at highest risk of iron deficiency anemia [4]. The mean age of children in this study was 5.67 years, the age range with the highest risk of anemia as reported by Calis et al [4]. When compared to the other two communities studied, Evbuomeore had a higher prevalence of anemia. The mean age of children in this community was 2.75 years, and this may explain the reduced hemoglobin concentration in these children, most of whom are in rapid growth spurts. This high rate may be indicative of the fact that the diet of the preschoolers is not adequate for their iron needs. During these studies, interviews performed with the children and observations by the community health workers revealed that the staple food of this area is garri. Farming is the major occupation and only crops that are likely to yield some income are planted. The level of income of family breadwinners is also low, as evidenced by the housing and the yield from the farms. Financial access to meat and other adequate animal sources of iron is, therefore, very limited. Efforts by public health workers in the health center in Evbuomeore to encourage mothers to grow their own vegetable gardens have not met with much success. These reasons could account for the very high prevalence of anemia observed in the study area.

The prevalence of asymptomatic malaria parasitaemia was 33.3%. Malaria was more prevalent in the age group 1-5 years; this agreed with the report by Anumudu et al [7]. It is known that the age group 1-5 years is the most vulnerable to severe and complicated malaria since their immunity is still low [7]. The prevalence of Plasmodium falciparum in the pooled subjects of 33.3% agreed with previous reports by Ekpo et al [10], which examined malaria parasites in Kwara, Oyo and Ogun States with a pooled prevalence of 33.6% among young children. This prevalence was higher than that reported by Jeremiah et al [11] in Rivers State of Nigeria, although less than that reported in Ibadan [7]. This study demonstrated heterogeneities in malaria prevalence among the studied communities in agreement with observation from a previous report [12], where parasites rates ranged from 12.0% to 37.8% among school children in the communities of Moship-Zango and Manhia in Ghana. This study adds to the growing body of evidence that malaria transmission can vary widely across communities and highlights the importance of targeting interventions to

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**Table 3** Prevalence of malaria parasitaemia among children

| Communities  | Number Infected | Mean Parasite Density |
|--------------|-----------------|-----------------------|
| Isiohor      | 60(42.5)        | 460                   |
| Evbuomeore   | 8(25.0)         | 465                   |
| Ekosodin     | 38(26.8)        | 463                   |

**Table 4** Malaria parasitaemia in relation to hemoglobin concentration

| Malaria Parasitaemia | Anemic (Hb<10 g/dL) | Non-Anemic (Hb>10g/dL) |
|----------------------|---------------------|------------------------|
| No. Infected (%)     | No. Infected (%)    |
| Malaria Parasitaemia | 52(49.1)            | 54(50.9)               |

P=0.06

**Table 5** Types and degree of malnutrition

| Type of malnutrition | Number. (%) |
|----------------------|-------------|
| Stunting             | 117(37.0)   |
| Wasting              | 61(19.3)    |
| Underweight          | 139(44.0)   |
specific communities [12].

Anemia accounts for up to one half of malaria deaths in young children [13]. It is evident that malaria causes anemia through one or more several mechanisms including blood loss, hemolysis, anemia of inflammation and splenic sequestration [14]. In this study, there was no association between malaria infection and anemia. Similarly, Stoltzfus et al [15] did not detect any association between malaria and anemia. In a recent study in River State, Nigeria, with school children as subjects, no association between malaria and anemia was found [11]. The non-association in this study and those of others may be attributed to low level parasitaemia observed in most of the infected children, a condition that is usually associated with concomitant immunity with little or no adverse effects on hemoglobin concentration.

The most common form of malnutrition in the children studied was underweight (44.0%) followed by stunting (37.0%) and wasting (19.3%). The prevalence of stunting in this study (36%) was similar to that estimated in the 4th World Nutrition Situation Report for the West African sub-region (34.9%). The estimated prevalence of underweight in this study was higher than the current rate for West Africa (36.5%) (16). Even though underweight affects fewer children globally than stunting (16), West Africa has seen an increase of 0.32% per year in recent years. Wasting was not as common as stunting and underweight in any region of the world, and a similar pattern was observed in the results of this study. West Africa has a wasting prevalence of 15.5% and in this study, 19.3%. There has been a substantial increase in wasting among West African children, and this increase could explain the high rate of underweight in these countries.

Chronic malnutrition appeared to be a more pressing problem than acute malnutrition as indicated by the levels of stunting and underweight compared to the levels of wasting, and the higher prevalence of anemia in preschool children than in school aged children. It is necessary, therefore, to educate mothers in this study area on the importance to appropriately feed their children from an early stage of life. There were significant differences in the MUAC of normal children and wasting and underweight children, suggesting that MUAC could be accurately used for rapid assessment of on-going malnutrition in specific populations, since it will be easier to establish local standards. The significant correlation between MUAC and PCV, underweight and wasting also supported this argument.

As seen in this study, serum ferritin levels, as measured by ELISA, can be reliably used to diagnose anemia. It has also been shown that nutritional status can modulate malaria morbidity and mortality [17]. There was no association between serum ferritin values and the presence of malaria parasites in this study; this suggests that the anemia measured in this population may have been due mostly to nutritional status. This conclusion was confirmed by work done in Lagos [17], which showed that ferritin levels were significantly higher in subjects with high densities of malaria parasites.

The most likely explanation for the high rate of anemia in this population is, therefore, insufficient dietary intake of micronutrients, especially iron. It may explain a delayed recovery of iron deficiency anemia after the control of parasitic infections. Iron supplementation through existing malaria control programs should be considered. Hemoglobinopathies and thalassaemia may also contribute to anemia. Attempts to investigate this in the current study have not been successful thus far. The effect of helminthiasis on anemia status in these rural communities has been studied and will be reported in a later study.

**Conclusion**

In rural communities of Edo State, Nigeria, anemia is highly prevalent; however, it cannot serve as a proxy indicator of malaria infections. This is likely caused by the marginal nutritional status of those at risk for malaria infection. Infection control should be accompanied by monitoring of iron deficiency and, when needed, supplementation can be organized through a malaria control program.

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