Malarial iron-deficiency anaemia among asymptomatic Nigerian children

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Abstract

Purpose. There is widespread subclinical (asymptomatic) malaria in our locality. The effects exerted by malaria on the body iron status remain incompletely understood. The aim of this study was to investigate the prevalence of malarial iron-deficiency anaemia and the effect of asymptomatic malaria on iron status indicators.

Design. A cross-sectional prospective study.

Methods. Seven parameters, haemoglobin concentration, white blood cell (WBC) count, malaria parasite, serum iron, total iron binding capacity (TIBC), serum ferritin, and percentage transferrin saturation, were evaluated among 240 children of both genders, aged 1–8 years. Estimation of the variables was carried out using standard haematological, colorimetric and enzyme-linked immunosorbent assay procedures.

Results. Of the 240 children surveyed, 66 (27.5%) were parasitized with Plasmodium falciparum. The overall prevalence of iron-deficiency anaemia (defined as haemoglobin <11 g dl⁻¹, serum ferritin <12 ng ml⁻¹ and transferrin saturation <16%) in this study population was 9/240 (3.75%). The prevalence of iron-deficiency anaemia among the parasitized children was 9/66 (13.6%). Their mean parasite density (3.35 × 10³ parasites ml⁻¹) was higher than the mean parasite density of the entire study population (1.16 × 10³ parasites ml⁻¹). No significant change in the haemoglobin concentration, WBC and serum ferritin values was observed between the parasitized and non-parasitized children (p>0.05), whereas a marked decrease in the serum iron, TIBC and percentage transferrin saturation values in the parasitized children was observed when compared with the non-parasitized group (p<0.02, 0.02 and 0.01). The percentage transferrin saturation correlated directly and significantly with haemoglobin, serum iron and TIBC values (r=0.317, 0.617, 0.236; p<0.01, p<0.01 and p<0.05). The reduction in the haemoglobin concentration, serum ferritin and percentage transferrin saturation values became evident when age was introduced into the analysis, with the children below 5 years of age more affected.

Conclusions. We conclude that: (1) asymptomatic malaria infection exerts significant effects on iron indicators; (2) an increase in transferrin saturation may be an indication of iron availability and vice versa; (3) children younger than 5 years of age constitute a high-risk group in malaria-endemic regions of developing countries; (4) there was a high prevalence of asymptomatic malaria and a low prevalence of iron-deficiency anaemia.

Key words: Iron-deficiency anaemia, malaria, iron status indicators, P. falciparum, Nigeria
Introduction

Anaemia is defined as a haemoglobin concentration lower than the established cut-off defined by the World Health Organization (WHO). This cut-off ranges from 110 g l\(^{-1}\) for pregnant women and for children 6 months–5 years of age to 120 g l\(^{-1}\) for non-pregnant women, to 130 g l\(^{-1}\) for men [1]. Anaemia is one of the most widespread public health problems, especially in developing countries, and a major cause of morbidity and mortality in malaria-endemic areas of sub-Saharan Africa [2].

WHO statistics show that 30–90% of children under 5 years of age in malaria-endemic areas have anaemia, 5–15% of severe anaemia in children under 5 years in endemic areas is due to malaria and 8–15% of child deaths are caused by severe anaemia due to malaria [2]. Most of the anaemia in children in malaria-endemic areas is complicated by nutritional iron deficiency, which in turn exerts a negative impact on the iron balance resulting in iron deficiency. Between 2 and 5 million people are at least mildly iron deficient, making iron deficiency the most common micronutrient deficiency in humans [3]. Iron deficiency, according to Van den Broek [4], is also believed to be the main underlying cause of anaemia. Although malaria causes a large proportion of anaemia in malaria-endemic areas, the contribution of underlying micronutrient malnutrition, creating a complex web of interactions with serious health repercussions, cannot be oversimplified. Micronutrient deficiencies have been associated with increased morbidity and mortality from malaria and malaria in turn may contribute to poor nutritional status, reflecting the classic vicious cycle of malnutrition and infection [4]. Iron status can be considered as a continuum from iron deficiency with anaemia, to iron deficiency with no anaemia, to normal iron status with varying amounts of stored iron and finally iron overload, which can cause organ damage when severe. Iron deficiency is the result of long-term iron imbalance, resulting in the absence of mobilizable iron stores and a compromised supply of iron to tissues, including the erythron. The more severe stage of iron deficiency is nearly always associated with anaemia [5,6].

In developing countries such as Nigeria, iron deficiency is generally the major cause of anaemia. Studies in Cote d’Ivoire [7] and Benin [8] estimated that iron-deficiency anaemia accounted for approximately 50% of the anaemia observed. In the Cote d’Ivoire study, the proportion of anaemic individuals with iron deficiency varied by age and gender. Approximately 80% of the anaemic pre-school-age children had iron-deficiency anaemia, compared with 50% of the school-age children and women and 20% of the men. Malaria and other infections or inflammatory disorders contributed significantly to the high prevalence of anaemia, particularly in young children, but these infections and/or disorders and iron deficiency could not explain all of the anaemia cases.

We used cross-sectional data to investigate the malaria subjects and also the effect of malaria on the biochemical iron status of Nigerian children. A study like this is important because there are strong indications that despite several programmes on malaria control, the situation has not changed appreciably and nothing is known presently about the interactions of malaria and iron-deficiency anaemia in this particular setting. This study was specifically aimed at determining the prevalence of asymptomatic malaria and iron-deficiency anaemia and also to assess the association between these variables.

Subjects and methods

The study was conducted in Rumueme, located in the capital city of Port Harcourt, Rivers State, Nigeria. The geographical location of Rivers State is latitude 4°31’–5°31’ and
longitude 6°30′–7°21′. Malaria transmission is throughout the year in this area due to the proximity to a swampy creek and poor environmental sanitation. Children were recruited between July 2005 and April 2006. There was no record of a previous survey of this nature conducted in this area and the prevalence of malaria and anaemia (haemoglobin concentration <11.0 g dl$^{-1}$) was not known. There is also no active malaria control programme in this area and no other epidemiological or entomological studies of malaria have been conducted previously.

A research laboratory in a clinic setting was established in the area and children were recruited from neighbouring households within the study area. The study received ethical approval from the Rivers State University of Science and Technology, Port Harcourt, Nigeria. Informed consent was obtained from the parents of participating children, in accordance with the Helsinki protocol. Children included in the study received free laboratory tests and medical care for anaemia and malaria. In total, 240 children (boys=117, girls=123, ratio 1:1.05) participated in the study.

**Design and procedures**

This study was purely cross-sectional and prospective in nature. Children were randomly selected and the selection of households for inclusion in this study was based on a random cluster sampling of the households identified within the prescribed area. The households were visited and the purpose of the study made known to them. The parents later brought their children to the research base after giving informed consent.

The eligibility criteria were: age 1–8 years; axillary temperature $\leq 37.5^\circ$C; absence of symptoms suggestive of malaria, anaemia or any systemic illness; parental consent given.

A sample of 2 ml of venous blood was collected into an ethylenediaminetetraacetic acid (EDTA) bottle for malaria and haematological investigations. Three millilitres of clotted blood was spun and the serum used for biochemical studies.

**Laboratory measurements**

The haemoglobin concentration was determined using the cyanmethylhaemoglobin method as recommended by the National Committee for Clinical Laboratory Standards [9]. Haemoglobin reagents from Pointe Scientific (USA) (catalogue no. 47504-500) were used for the determination.

Serum iron, total iron binding capacity (TIBC) and transferrin saturation were estimated using a ferrozine-based iron/TIBC reagent set (Pointe Scientific; lot no. 516703). Test procedures were followed as contained in the manufacturer’s standard operating manual inserted in the kit. Transferrin saturation was calculated from the serum iron concentration and TIBC values as follows: serum iron/TIBC $\times 100$.

The measurement of serum ferritin was carried out using the human ferritin enzyme immunoassay test kit (Pointe Scientific; lot no. RN 23128). The ferritin quantitative test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay was carried out on an ELISA machine (STAT FAX 2100, Awareness Technology). Test procedures were followed as contained in the manufacturer’s standard operating manual. Reading was carried out using point-to-point mode, from an analogue printer (Epson LX 300+).

Malaria was estimated by microscopy with well-stained Giemsa smears (thick and thin) using 100 $\times$ oil immersion. Thick and thin blood smears were stained with fresh working Giemsa stain according to standard procedures. Parasite densities were recorded as a ratio.
of parasites to 500 white blood cells (WBCs) from thick smears. Density (parasites $\mu l^{-1}$) = parasites/500 WBCs × WBC count of individual subjects.

The WBC count was carried out by diluting well-mixed whole blood with Turk's solution in the ratio of 1:20 (950 $\mu l$ Turks solution to 50 $\mu l$ whole blood). The haemocytometer was then filled with an aliquot of this mixture and allowed to settle for 1 min before performing the count. WBCs were counted with $\times 10$ objective with reduced light in the four corner squares of the counting chamber. The number of WBC mm$^3$ was calculated as follows: cells counted × dilution factor × chamber depth/area of chamber counted. For example, if 100 WBCs were counted in four large corners of a 1 mm square with a depth of 0.1 mm, the WBC count would be $100 \times 20 \times 10^6/0.4 = 25 \times 20 \times 10^6 = 5000 \times 10^6 = 5.0 \times 10^9 l^{-1}$.

Statistics

Data were arranged in a 2 × 2 contingency table and analysed using the Statistical Package for Social Sciences (SPSS) (version 11.0, Chicago, IL, USA). The non-parametric test (Kruskal–Wallis) was used for the analysis of skewed data, whereas the t-test was used for parametric data.

The biochemical iron status was expressed as median and interquartile ranges, whereas haemoglobin and WBC values were expressed as mean and standard deviation. Boxplots were used to show the influence of malaria on biochemical parameters.

Results

Two hundred and forty children were surveyed, 66 of them were found to be parasitized with Plasmodium falciparum, giving a prevalence rate of 27.5%. The parasitized group was further divided according to gender and age groups. The mean and median values of the iron-deficiency anaemia indicators according to gender and age groups of participating children are shown in Tables I and II. Haemoglobin, TIBC, serum ferritin and transferrin saturation values were lower in boys than in girls, whereas serum iron values were lower in girls ($p < 0.05$).

Table I. Mean and median values of the parameters among parasitized and non-parasitized children according to gender.

<table>
<thead>
<tr>
<th></th>
<th>Parasitized children (n=66)</th>
<th>Non-parasitized children (n=174)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys</td>
<td>Girls</td>
</tr>
<tr>
<td>Haemoglobin g dl$^{-1}$</td>
<td>9.8 ± 2.3*</td>
<td>11.2 ± 1.4</td>
</tr>
<tr>
<td>(mean; SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum iron µg dl$^{-1}$</td>
<td>34.4 (17.2–83.8)</td>
<td>44.4 (17.2–103.9)*</td>
</tr>
<tr>
<td>(median; IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIBC µg dl$^{-1}$</td>
<td>206 (158.7–391.7)</td>
<td>264.5 (113.9–483)**</td>
</tr>
<tr>
<td>(median; IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum ferritin ng ml$^{-1}$</td>
<td>23.5 (8.6–38.9)</td>
<td>55.6 (36.1–70.2)*</td>
</tr>
<tr>
<td>(median; IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transferrin saturation %</td>
<td>17.2 (11.8–24.5)</td>
<td>16.8 (14.3–20.7)$^{ns}$</td>
</tr>
<tr>
<td>(median; IQR)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD, standard deviation; IQR, interquartile range; TIBC, total iron binding capacity; ns, not significant.

*p < 0.05; **p < 0.01.
Similarly, haemoglobin, serum ferritin and transferrin saturation values were lower in parasitized children below 5 years of age. TIBC was decreased significantly in the older group. There was no change in serum iron values between the two groups.

Generally, the effect of malaria infection on median values of the biochemical iron indicators in the study population represented as boxplots (Figures 1–4) revealed significant changes in serum iron, TIBC and transferrin saturation values.

Table II. Mean and median values of the parameters among parasitized and non-parasitized children according to age groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parasitized children (n=66)</th>
<th>Non-parasitized children (n=175)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;5 years</td>
<td>5–8 years</td>
</tr>
<tr>
<td>Haemoglobin g dl(^{-1})</td>
<td>9.2±2.4*</td>
<td>11.8±1.3</td>
</tr>
<tr>
<td>Serum iron µg dl(^{-1})</td>
<td>43.1 (17.2–120.1)</td>
<td>43.1 (17.2–89.0)*</td>
</tr>
<tr>
<td>TIBC µg dl(^{-1})</td>
<td>267.0 (150.2–669.4)</td>
<td>181.5 (113.9–483)**</td>
</tr>
<tr>
<td>Serum ferritin ng ml(^{-1})</td>
<td>33.5 (12.0–48.1)**</td>
<td>39.2 (19.2–57.2)</td>
</tr>
<tr>
<td>Transferrin saturation %</td>
<td>13.0 (10.5–21.5)</td>
<td>20.0 (16.5–35.0)</td>
</tr>
</tbody>
</table>

SD, standard deviation; IQR, interquartile range; TIBC, total iron binding capacity; ns, not significant. *\(p<0.05\); **\(p<0.01\).

Figure 1. Effect of malaria parasite on serum iron concentrations.
Figure 2. Effect of malaria infection on total iron binding capacity concentrations.

Figure 3. Effect of malaria infection on serum ferritin concentrations.
The prevalence of iron-deficiency anaemia (defined as haemoglobin concentration <11 g dl$^{-1}$, serum ferritin <12 ng ml$^{-1}$ and transferrin saturation <16%) in the study population was 9/240 (3.75%). The prevalence of iron-deficiency anaemia among malaria parasitized children was high 9/66 (13.6%). Their parasite density ($3.35 \times 10^3$ parasite$\mu l^{-1}$) was found to be higher than the parasite density of the entire study population ($1.16 \times 10^3$ parasites$\mu l^{-1}$). There was no change in the haemoglobin and WBC count values between the parasitized and non-parasitized group (Table III).

Table IV shows Pearson bivariate correlation of the variables used in this study. Parasite density is significantly and negatively related to serum ferritin values ($p<0.05$). A positive relationship exists between haemoglobin values and transferrin saturation ($p<0.01$), serum iron, TIBC and transferrin saturation values ($p<0.01$).

Table III. Prevalence of iron-deficiency anaemia, haemoglobin values, white blood cell count and parasite densities in the study population.

<table>
<thead>
<tr>
<th></th>
<th>Parasitized (n=66)</th>
<th>Non-parasitized (n=174)</th>
<th>Overall (n=240)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron-deficiency anaemia*</td>
<td>6 (9.0%)</td>
<td>3 (1.72%)</td>
<td>9/240 (3.75%)</td>
</tr>
<tr>
<td>Haemoglobin (g dl$^{-1}$)</td>
<td>$11.12 \pm 1.9$</td>
<td>$11.23 \pm 1.8$</td>
<td>$11.2 \pm 1.8$</td>
</tr>
<tr>
<td>Parasite density ($\times 10^3$ parasite$\mu l^{-1}$)†</td>
<td>$1.16 \pm 0.88$</td>
<td>–</td>
<td>$1.16 \pm 0.88$</td>
</tr>
<tr>
<td>White blood cell count ($\times 10^9$ l$^{-1}$)</td>
<td>$5.4 \pm 2.3$</td>
<td>$5.3 \pm 2.3$</td>
<td>$5.1 \pm 2.0$</td>
</tr>
</tbody>
</table>

*Defined as haemoglobin <11 g dl$^{-1}$, serum ferritin <12 ng ml$^{-1}$ and transferrin saturation <16%.
†Parasite density of the 9% iron-deficient anaemic subjects was $4.45 \times 10^3$ parasites$\mu l^{-1}$. 
**Table IV.** Pearson correlation of the variables used in this study.

<table>
<thead>
<tr>
<th>Parasite density</th>
<th>Haemoglobin</th>
<th>Serum iron</th>
<th>Serum ferritin</th>
<th>TIBC</th>
<th>Transferrin saturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000</td>
<td>0.370</td>
<td>0.172</td>
<td>-0.450*</td>
<td>-0.260</td>
<td>-0.231</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.161</td>
<td>-0.012</td>
<td>0.080</td>
<td>0.317**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.000</td>
<td>-0.051</td>
<td>0.786**</td>
<td>0.617**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
<td>0.178</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
<td>0.236*</td>
</tr>
</tbody>
</table>

*pCorrelation is significant at the 0.05 level (p<0.05); **correlation is significant at the 0.01 level (p<0.01).
TIBC, total iron binding capacity.

**Discussion**

Many African children presenting to hospitals with severe, acutely life-threatening anaemia have no symptoms or recent history of symptoms of malaria [10]. Our findings prospectively show that the prevalence of *P. falciparum* malaria among these asymptomatic children was high (27.5%) and that of malarial iron-deficiency anaemia was also high (13.6%). A low parasitaemic level (1.16 × 10^3 ml⁻¹) in the study population was observed. It appears that at least in a large proportion of these children, severe anaemia has developed following prolonged exposure to chronic or repeated infection with low levels of parasitaemia. Likewise, reports from many community-based surveys [4,7,8] in malaria-endemic areas indicate that a substantial proportion of children have severe anaemia. The vast majority of these children are asymptomatic and they often have low-level parasitaemia. These observations are in agreement with the results obtained from this study.

Malaria causes a shift of iron distribution from functional towards storage compartments [11], which has also been observed in a range of other infections [12–16]. Thus, in malaria, stainable macrophage iron stores are often present in bone marrow [17,18] and serum ferritin concentrations are increased [11,17,18]. Ferritin is an iron storage protein, but its serum concentration also increases in infection, probably as part of a host immune response [19]. Serum ferritin concentrations <12 ng ml⁻¹ are highly predictive of depleted iron stores [20], whereas values above this range, when coupled with anaemia, indicate inflammation, but may mask co-existing iron deficiency [21].

In this study, the presence of inflammation could not be confirmed due to a lack of facilities, but iron deficiency on the basis of ferritin concentrations ≤16 ng ml⁻¹ alone was observed in 13.75%, which was quite high in the study population. A combination of haemoglobin concentration, ferritin concentration and transferrin saturation was used to define iron-deficiency anaemia in this population and it was observed that the prevalence was low (3.75%). Thus, it could be deduced that although severe anaemia is a prominent feature of acute malaria infection, a low prevalence of iron-deficiency anaemia characterizes asymptomatic malaria infection.

Anaemia and the shift in iron distribution from functional to storage compartments are observed in malaria and a range of other infections. This suggests that the pathogenic mechanisms underlying the anaemia of chronic disease also play a role in the development of malarial anaemia [19,21,22].

The anaemia of chronic disease is primarily due to reduced erythropoietin production and reduced responsiveness of erythroid progenitor cells to erythropoietin [12,13] under...
the influence of pro-inflammatory erythropoietin, such as tumour necrosis factor and interleukin-1. Therapy using recombinant erythropoietin can correct the anaemia of chronic disease but not of iron deficiency [12]. Thus, the distinctive abnormalities in body iron distribution appear to be a by-product of these mechanisms [23]. It cannot be ruled out, however, that some iron sequestration occurs in malaria in hemazoin, a product from haemoglobin degradation by malaria parasites that is found in circulating or phagocytosed red cells.

In this study, serum transferrin receptor was not measured to assess the anaemia of iron deficiency and erythropoiesis, but the analysis of the effect of malaria on iron markers revealed a significant reduction in the serum iron, TIBC and transferrin saturation and normal serum ferritin concentration among the malaria-infected subjects. This finding is consistent with anaemia of chronic disorders [23]. In a correlation study, transferrin saturation was found to be positively and significantly associated with the haemoglobin concentrations \( (p < 0.01) \). This implies that as the transferrin saturation drops, the haemoglobin concentration also drops and vice versa. Thus, if malaria exerts an effect on the transferrin saturation, the haemoglobin concentration will equally be affected. In other words, increased transferrin saturation indicates iron availability. An inverse relationship was found to exist between parasite densities and serum ferritin concentrations. This implies that as the malaria parasite increases in density, there will be a concomitant decrease in the serum ferritin values. This could be true of severe falciparum malaria. In this study with asymptomatic children, we did not observe any significant decrease in serum ferritin concentrations among the parasitized children. Serum ferritin values were not significantly different in both malaria parasitaemia-positive and -negative subjects, whereas serum iron, TIBC and transferrin saturation values were significantly lower in parasitaemic subjects. These observations are contrary to the findings of Odunukwe et al. [24,25] in which the serum ferritin level was reported to be increased in asymptomatic malaria parasitaemia.

When adjusted for age, a significant reduction was observed in the haemoglobin concentration, serum ferritin and transferrin saturation among parasitized children below 5 years of age, whereas a significant decrease in the TIBC values was observed among parasitized children aged 5–8 years old. Thus, it could be deduced that the impact of asymptomatic malaria infection on iron-deficiency anaemia is felt most among children below 5 years of age. This observation is consistent with several reports [26–29] that children under 5 years of age are more vulnerable to iron deficiency and iron-deficiency anaemia than older children and this calls for urgent intervention. A significant anaemia was noticed among parasitized boys rather than girls \( (p < 0.05) \). The reasons for this are not fully understood.

In summary, our findings indicate that in asymptomatic malaria [1] there are no changes in the haemoglobin concentrations and ferritin values [2]. There is a significant reduction in serum iron, TIBC and percentage transferrin saturation [3]. Transferrin saturation is directly significantly correlated with haemoglobin concentration, i.e. increased transferrin saturation implies availability of iron [4]. A decrease in haemoglobin, serum ferritin and transferrin saturation is associated with age, thus children below 5 years of age are more vulnerable to iron-deficiency anaemia in asymptomatic malaria infection.

References
