Relationship between Carotenoids and Anaemia during Acute Uncomplicated *Plasmodium falciparum* Malaria in Children

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ABSTRACT

A clinic-based cohort study in Kampala, Uganda, was conducted to examine the relationship between severe malarial anaemia and plasma micronutrients. Plasma carotenoids, retinol, vitamin E, and four trace metal concentrations were measured at enrollment and seven days later in 273 children, aged 1-10 year(s), with acute, uncomplicated *Plasmodium falciparum* malaria. Concentrations of plasma provitamin A carotenoids (p<0.0001), non-provitamin A carotenoids (p<0.0001), retinol (p<0.0001), all four trace elements (all p<0.001), and vitamin E (p<0.0001) rose significantly by day 7 among children without severe anaemia (haemoglobin 70 g/L). There was no change in provitamin A carotenoids (p=0.24) among children with severe anaemia (haemoglobin <70 g/L), whereas non-provitamin A carotenoids (p<0.0001), retinol (p<0.0001), and vitamin E (p=0.011) increased. These observations also support the hypothesis that the use of provitamin A carotenoids increases during malaria infection.

Key words: Anaemia; Malaria; *Plasmodium falciparum*; Carotene; Cryptoxanthin; Lycopene; Lutein; Zeaxanthin; Retinol; Tocopherol; Cohort studies; Uganda

INTRODUCTION

Anaemia is one of the most common and severe outcomes of infection with *Plasmodium falciparum*. It is a significant complication of malaria not only in children with acute febrile illness but also in those with asymptomatic falciparum infection (1-3). In Kenya, twice as many patients admitted with falciparum malaria are cases of malarial anaemia compared to cerebral malaria (4). In areas with perennial transmission of malaria, severe anaemia may be more common than cerebral malaria (5). Although its serious impact during malaria has not been fully assessed, anaemia is almost always present (6) and is recognized as an important cause of death in endemic areas.

In endemic areas, such as in Uganda, patients presenting with malaria are generally children who are anaemic when first seen (4) and with a history of malarial symptomatology spanning several weeks. This picture of chronic illness is unlike the one seen in patients suffering from severe malaria, who become anaemic over the course of the infection and subsequent to their illness (7).

The mechanisms involved in the occurrence of anaemia resulting from malaria are still unclear but have been shown to involve non-immune and immune-mediated haemolysis, decreased red cell production, and dyserythropoiesis (4). Cytokines, such as tumor necrosis
factor (TNF-α), interleukin-10 (IL-10), and acute phase proteins, such as α1-acid glycoprotein (Nussenblatt, unpublished data) may play a role in malarial anaemia (8,9).

In many areas endemic for malaria, high rates of malnutrition and micronutrient deficiencies are also observed. Several of these micronutrients, including vitamin A and E and carotenoids and zinc, have essential roles in immune function (10-13) and are implicated in resistance to many infectious diseases (13-15). Increased importance has been given to the role that nutrition plays in malaria, and vitamin deficiencies have been associated with morbidity and mortality due to malaria. Individuals with malaria have lower plasma concentrations of several micronutrients compared to controls (16-20). Supplementation of vitamin A to children reduces morbidity due to malaria by 30% (21), and supplementation of zinc resulted in 38% reduction in attendance in a Plasmodium falciparum health centre by preschool children (22).

The purpose of our study was to examine the relationship between concentrations of serum carotenoids, retinol, vitamin E, and four trace elements and the severity of anaemia in children with acute, uncomplicated falciparum malaria in Kampala, Uganda.

MATERIALS AND METHODS

The study population consisted of a consecutive sample of children, aged 1-10 year(s), seen in the acute paediatric care unit of Mulago Hospital, Kampala, Uganda, during August-December 1998. The Mulago Hospital serves urban and peri-urban Kampala, an area endemic for Plasmodium falciparum malaria. Children were eligible for the study if they were aged 1-10 year(s), were positive for malaria on the basis of a thick smear, had haemoglobin >50 g/L, were not admitted for transfusion, and had no evidence of cerebral malaria.

Upon presentation at the acute care unit of the hospital, the children were seen by a medical officer. Temperature was recorded using an oral thermometer. A fingerstick blood sample was taken to prepare thick and thin blood films to determine the presence or absence of malarial parasites, level of parasitaemia, and concentrations of haemoglobin using a haemoglobinometer (HemoCue Inc, Mission Viejo, CA, USA) (23). Height and weight were measured using a child-measuring board (Shorr Productions, Olney, MD) and a digital scale respectively.

Children with malaria were enrolled in the study after written, informed consents were obtained from the parents or guardians. The entire consent form was read to the parent or guardian in the local language Luganda, or in English, as appropriate, and participants were given a copy of the consent form after signature or thumbprint was obtained. A medical officer examined children at enrollment and on day 3 and 7, and spleen enlargement was assessed using the Hackett scale (24).

Standard anthropometric techniques were used for measuring length and weight of children (25), and growth standards of the National Center for Health Statistics were used as reference (26). Weight-for-age z-score <-2, weight-for-height z-score <-2, and height-for-age z-score <-2 were considered consistent with underweight, wasting, and stunting as per convention (26). At enrollment and on day 3 and 7, a venous blood sample was collected by venipuncture (Sarstedt Monovette, Newton, NC). Blood samples were shielded from bright light and immediately aliquoted and stored in cryotubes at -70°C. All children were treated with chloroquine sulphate as per guidelines of the Ugandan Ministry of Health. Children with increased parasitaemia on day 3 or day 7 were treated with quinine or fansidar. The study protocol was approved by the Joint Committee for Clinical Investigation of Johns Hopkins School of Medicine, the Mulago Hospital Ethical Committee, and the Ugandan National Council of Science and Technology.

Thick and thin giemsa-stained blood films were analyzed for the number of parasites per 200 white blood cells. Slides were considered negative if no parasites were seen in 100 fields on the thick film. Plasma α1-acid glycoprotein was measured using radial immunodiffusion (Bindarid, The Binding Site, Birmingham, UK) with a between-assay coefficient of variation of 10.3%. C-reactive protein was measured by enzyme-linked immunosorbent assay (Virgo CRP 150, Hemagen Diagnostics, Waltham, MA, USA) with a between-assay coefficient of variation of 8.6%.

Concentrations of plasma α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin (not separated with this procedure), retinol, α-tocopherol, and γ-tocopherol for baseline and day 7 were determined by high-performance liquid chromatography (27). The internal standards used were tocol (Hoffmann-La Roche, Nutley, NJ) at 320 nm and all-trans-ethyl-β-apo-8’-carotenode (purified sample, courtesy: Dr. Fred Khachik, USDA) at 450 nm. Between-run coefficients of variation
for pooled standards were 5.6% for retinol, 5.4% for α-carotene, 7.6% for β-carotene, 7.3% for β-cryptoxanthin, 11.5% for lycopene, 5.0% for lutein/zeaxanthin, 12.6% for α-tocopherol, and 8.6% for γ-tocopherol. Quality control was assessed by repeated analysis of standard reference material (SRMb, National Institute of Standards and Technology, Gaithersburg, MD) and pooled reference standards. All samples were run in a masked fashion.

Plasma concentrations of trace elements were measured using a Perkin Elmer AAnalyst 600 atomic absorption spectrophotometer equipped with Zeeman background correction, a THGA graphite furnace, and an AS800 autosampler (28). An electrodeless discharge lamp (EDL) and a pyrolytically-coated graphite furnace tube with an integrated L’vov platform were used for detection of selenium, whereas a Lumina hollow cathode lamp (HCL) and a standard THGA tube with an integrated L’vov platform and endcaps were used for detection of copper, iron, and zinc (Perkin Elmer Corp., Norwalk, CT, USA).

Plasma samples were thawed and diluted four-fold for selenium analysis by adding 50 µL of plasma to 50 µL of H₂O and 100 µL of a 0.17 M HNO₃ (Optima Grade, Fisher, Pittsburgh, PA, USA), 0.1% TritonX100 (SigmaUltra, Sigma Chemical, St. Louis, MO, USA) solution (29). Fifteen µL of the diluted plasma was injected into the furnace tube with 5 µL of 1 mg/mL Pd(NO₃)₂, 0.6 mg/mL Mg(NO₃)₂ (Perkin Elmer) as a modifier. For analysis of copper and iron, the plasma was ultimately diluted 20-fold by adding 50 µL of the previously-diluted sample to 200 µL of H₂O. Fifteen µL of this sample was then injected into the furnace (30,31). Sample cups with slotted lids were used for minimizing evaporation during the sample runs (Sarstedt, Newton, NC). Two pooled human serum controls were run at the beginning and end of each batch of samples to determine within and between-run coefficients of variation. Analysis of zinc required that the plasma be diluted 200-fold using 5 µL of the thawed sample and 995 µL of de-ionized distilled water. Fifteen µL of the diluted sample was injected into the furnace with 5 µL of 1 mg/mL Mg(NO₃)₂. “Seronorm” trace elements serum (Accurate Chemical and Scientific Corp., Westbury, NY, USA) and zinc standard (NIST, Rockville, MD) were used as reference materials (32). The between-run coefficient of variation was 6.35% for selenium analysis, 9.90% for copper, 11.98% for iron, and 8.75% for zinc.

At baseline, children were separated into two groups according to concentrations of their haemoglobin. Children with haemoglobin <70 g/L were classified as having severe anaemia and those with haemoglobin ≥70 g/L were classified as not having severe anaemia. Since parasitaemia was highly skewed toward higher values, it was transformed by log₁₀ to normalize its distribution. The differences in age, log₁₀ parasitaemia, and anthropometric indices at baseline were compared according to status of anaemia using a two-sample t-test. The differences in analyte levels on day 7 and baseline were assessed within each group using a paired t-test. Multiple linear regression was used for examining the relationship between analyte level and initial status of anaemia at baseline and on day 7. The regression equation for both the days was analyte concentrations (μmol/L)=β₀+β₁(severely anaemic)+β₂ (age)+β₃ (weight-for-height z). For β₁, 1=severely anaemic and 0=not severely anaemic. Multiple linear regression was used for assessing the relationship between concentrations of haemoglobin at baseline and the change in concentrations of provitamin A and non-provitamin A between baseline and day 7, using haemoglobin as a continuous variable.

**RESULTS**

Of the 273 children, 21% had severe anaemia with concentrations of haemoglobin <70 g/L. Children with severe anaemia were significantly younger, with a mean (±SD) age of 29.5 months (26.1) compared to 40.4 months (26.1) for children with haemoglobin ≥70 g/L (p=0.005). Parasitaemia at baseline was not associated with the severity of anaemia (p=0.611). Table 1 shows the baseline characteristics of the severely-anaemic children versus the children without severe anaemia. The mean haemoglobin level (±SD) for the severely-anaemic children was 62.4 (5.7) compared to 93.1 (15.9) for the children without severe anaemia (p<0.0001). The only anthropometric measurement that differed between the two groups was the weight-for-height z-score (p=0.009). At baseline, concentration of α₃-acid glycoprotein (AGP) was higher in the severely-anaemic children (p=0.0014), and concentration of C-reactive protein was not significantly different between the two groups of children (data not shown). Fifty children, who had increased parasitaemia on day 3, were considered to have treatment failures and were given quinine.

On day 7, serum concentrations of β-cryptoxanthin, lycopene, lutein/zeaxanthin, retinol, α-tocopherol, and...
αₐ-carotene and β-carotene on day 7 than at baseline, while concentrations of these analytes did not show a significant increase by day 7 for the severely-anaemic children. Concentrations of iron, selenium, and zinc were also higher by day 7 in both the groups, but concentrations of copper only increased significantly by day 7 in the children who were not severely anaemic (Table 2).

Table 3 compares the differences in serum concentrations of retinol, carotenoids, vitamin E, and trace elements at baseline and day 7 between the children who were not severely anaemic and those who were severely anaemic at baseline. Results of multiple linear regression showed no significant differences in any provitamin A carotenoids between the two groups at baseline, after adjustment for age, and weight-for-height z-score. The children who were severely anaemic, however, had significantly lower concentrations of total non-provitamin A carotenoid. Concentrations of lutein/zeaxanthin in these children were lower (p<0.05), while the lycopene levels were not significantly different from those of the other children. Concentrations of γ-tocopherol and vitamin E at baseline were significantly different according to anaemia status. The children who were severely anaemic had higher concentrations of vitamin E compared to those who were not severely anaemic (p<0.01). On day 7, no differences remained in concentrations of vitamin E between the groups. Levels of copper at baseline were higher in children who were severely anaemic (p=0.001) and remained higher in those children on day 7 compared to the children who were not severely anaemic at baseline (p=0.003). When haemoglobin was assessed as a continuous variable, the difference in concentrations of provitamin A carotenoid between baseline and day 7 was significantly related to concentrations of haemoglobin at baseline (p<0.01), but the change in concentrations of non-provitamin A carotenoid was not significantly related to concentrations of haemoglobin at baseline (p>0.05).

**DISCUSSION**

In our study, the children who were severely anaemic at baseline were younger and more underweight than children with higher haemoglobin levels. This finding is consistent with those in other studies that show that severe malaria tends to involve severe anaemia mostly in children aged less than three years (33). We have previously found that there is a relationship between concentrations of haemoglobin and age of children with acute, uncomplicated malaria (Nussenblatt, unpublished data). While malnutrition and anorexia during infection can affect the concentrations of micronutrients, a study in Nigeria suggests that acute malaria is a more significant factor in lowering the plasma concentrations of antioxidant vitamins than is malnutrition (16). A significant correlation between the severity of anaemia and parasitaemia may have been observed had children with more severe malaria been enrolled. In addition to parasitaemia, other factors, such as immune haemolysis, may have contributed to anaemia of the children.

Several studies have shown lower serum concentrations of micronutrients in malaria patients...
Table 2. Analyte level at baseline and day 7 according to anaemia status at baseline

<table>
<thead>
<tr>
<th>Analyte*</th>
<th>Baseline</th>
<th>Day 7</th>
<th>p value†</th>
<th>Baseline</th>
<th>Day 7</th>
<th>p value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Severe anaemic, Hg &lt;70 (n=39)</td>
<td>Not severely anaemic, Hg ≥70 (n=148)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-carotene (µmol/L)</td>
<td>0.34±0.29</td>
<td>0.37±0.25</td>
<td>0.23</td>
<td>0.51±0.41</td>
<td>0.60±0.45</td>
<td>0.0001</td>
</tr>
<tr>
<td>β-carotene (µmol/L)</td>
<td>0.15±0.11</td>
<td>0.16±0.12</td>
<td>0.17</td>
<td>0.23±0.20</td>
<td>0.27±0.22</td>
<td>0.0001</td>
</tr>
<tr>
<td>β-cryptoxanthin (µmol/L)</td>
<td>0.057±0.054</td>
<td>0.056±0.050</td>
<td>0.80</td>
<td>0.82±0.086</td>
<td>0.94±0.12</td>
<td>0.086</td>
</tr>
<tr>
<td>Provitamin A carotenoids§ (µmol/L)</td>
<td>0.55±0.38</td>
<td>0.59±0.38</td>
<td>0.24</td>
<td>0.83±0.62</td>
<td>0.97±0.68</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lycopene (µmol/L)</td>
<td>0.12±0.086</td>
<td>0.14±0.096</td>
<td>0.031</td>
<td>0.14±0.10</td>
<td>0.18±0.12</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lutein/xaxanthin (µmol/L)</td>
<td>0.25±0.11</td>
<td>0.34±0.19</td>
<td>0.0001</td>
<td>0.33±0.20</td>
<td>0.40±0.20</td>
<td>0.0001</td>
</tr>
<tr>
<td>Non-provitamin A carotenoids‡ (µmol/L)</td>
<td>0.37±0.15</td>
<td>0.48±0.21</td>
<td>0.0001</td>
<td>0.48±0.26</td>
<td>0.58±0.28</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total carotenoids** (µmol/L)</td>
<td>0.92±0.51</td>
<td>1.07±0.53</td>
<td>0.0085</td>
<td>1.30±0.80</td>
<td>1.55±0.87</td>
<td>0.0001</td>
</tr>
<tr>
<td>Retinol (µmol/L)</td>
<td>0.61±0.23</td>
<td>1.24±0.50</td>
<td>0.0001</td>
<td>0.67±0.29</td>
<td>1.32±0.45</td>
<td>0.0001</td>
</tr>
<tr>
<td>α-tocopherol (µmol/L)</td>
<td>7.66±2.33</td>
<td>8.53±2.61</td>
<td>0.0032</td>
<td>7.48±2.30</td>
<td>8.65±2.63</td>
<td>0.0001</td>
</tr>
<tr>
<td>γ-tocopherol (µmol/L)</td>
<td>21.78±18.85</td>
<td>31.49±28.88</td>
<td>0.015</td>
<td>14.75±11.64</td>
<td>27.71±21.63</td>
<td>0.0001</td>
</tr>
<tr>
<td>Vitamin E§ (µmol/L)</td>
<td>29.44±18.85</td>
<td>40.02±29.46</td>
<td>0.011</td>
<td>22.23±1.25</td>
<td>36.35±22.66</td>
<td>0.0001</td>
</tr>
<tr>
<td>Copper (µg/L)</td>
<td>2429.30±84.30</td>
<td>2375.57±45.40</td>
<td>0.431</td>
<td>2176.47±42.30</td>
<td>2064.96±45.34</td>
<td>0.0009</td>
</tr>
<tr>
<td>Iron (µg/L)</td>
<td>663.03±82.56</td>
<td>987.01±102.51</td>
<td>0.008</td>
<td>677.23±63.04</td>
<td>975.26±61.85</td>
<td>0.0005</td>
</tr>
<tr>
<td>Selenium (µg/L)</td>
<td>55.33±2.67</td>
<td>65.05±3.02</td>
<td>0.0001</td>
<td>63.77±1.61</td>
<td>70.43±1.56   &lt;0.00001</td>
<td></td>
</tr>
<tr>
<td>Zinc (µg/L)</td>
<td>475.37±37.73</td>
<td>677.61±46.09</td>
<td>&lt;0.00001</td>
<td>456.59±15.29</td>
<td>646.39±15.12</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

* Mean±SD
† Paired t-test
§ α-carotene + β-carotene + β-cryptoxanthin
‡ Lycopene + lutein/xaxanthin
** α-carotene + β-carotene + β-cryptoxanthin + lycopene + lutein/xaxanthin
†α-tocopherol + γ-tocopherol
Nigerian children that found that malaria cases did not have a decline in plasma \( \alpha \)-tocopherol compared to haemoglobin may explain why concentrations of vitamin A did not differ in more anaemic children (5). Various explanations have been given for the reduced plasma concentration of retinol during malaria infection.

Retinol-binding protein (RBP) is an 18,000-Da protein that extravasates readily when vascular permeability is enhanced by the acute-phase response. One study showed that RBP and concentrations of retinol decreased in malaria patients and that RBP accounted for 94.6% of the variance in retinol (17). Thurnham and colleagues postulated that decline in serum concentrations of retinol during infection is indicative of a rapid distribution of retinol into extravascular fluids where these can more efficiently maintain tissues being exposed to reactive oxygen species resulting from the infection (41). In addition, the synthesis of acute-phase reactants may increase the need for retinol uptake since retinol may help incorporate mannose into glycoproteins during synthesis, and most acute-phase reactants are glycoproteins (41-43).

A similar explanation is offered for the decrease in concentrations of carotenoids in patients with malaria. Concentrations of carrier molecules of these micronutrients, such as lipoproteins, diminish during infection and may contribute at least partially to the low concentrations of carotenoids in malaria cases versus controls to become non-significant (37).

Adjustment for serum cholesterol concentration removes differences in patients with uncomplicated infections with a study in Thai patients showing that adjustment for cholesterol causes differences in \( \alpha \)-tocopherol in malaria cases versus controls to become non-significant (37).

Concentrations of vitamin A were not related to anaemia status at baseline. Vitamin A plays an important role in haematopoiesis (38,39). Hodges and colleagues observed that vitamin A deficiency in humans resulted in anaemia despite supplementation of iron (40). It has been thought that vitamin A deficiency may modulate stem cell division and differentiation and adversely affect haematopoiesis. Concentrations of vitamin A have also been shown to be correlated with acute-phase reactants during malaria infection (20). Since all the children in our study had acute malaria and inflammation, this could explain why serum concentrations of retinol were not significantly different in both the groups of children. In addition, a negative correlation has been reported between serum retinol and parasitaemia among children with asymptomatic infection (20). The lack of any relationship between parasitaemia and concentrations of

<table>
<thead>
<tr>
<th>Analyte (( \mu )mol/L)</th>
<th>Baseline</th>
<th></th>
<th>Day 7</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta )±SD</td>
<td>p value</td>
<td>( \beta )±SD</td>
<td>p value</td>
</tr>
<tr>
<td>( \alpha )-carotene</td>
<td>-0.06±0.054</td>
<td>0.264</td>
<td>-0.14±0.069</td>
<td>0.041</td>
</tr>
<tr>
<td>( \beta )-carotene</td>
<td>-0.043±0.025</td>
<td>0.084</td>
<td>-0.073±0.035</td>
<td>0.039</td>
</tr>
<tr>
<td>( \beta )-cryptoxanthin</td>
<td>-0.017±0.011</td>
<td>0.139</td>
<td>-0.037±0.019</td>
<td>0.061</td>
</tr>
<tr>
<td>Provitamin A carotenoids†</td>
<td>-0.12±0.078</td>
<td>0.138</td>
<td>-0.25±0.11</td>
<td>0.018</td>
</tr>
<tr>
<td>Lycopene</td>
<td>-0.007±0.013</td>
<td>0.598</td>
<td>-0.024±0.018</td>
<td>0.195</td>
</tr>
<tr>
<td>Lutein/zeaxanthin</td>
<td>-0.059±0.027</td>
<td>0.028</td>
<td>-0.034±0.035</td>
<td>0.335</td>
</tr>
<tr>
<td>Non-provitamin A carotenoids§</td>
<td>-0.066±0.033</td>
<td>0.048</td>
<td>-0.057±0.044</td>
<td>0.199</td>
</tr>
<tr>
<td>Total carotenoids‡</td>
<td>-0.18±0.10</td>
<td>0.073</td>
<td>-0.31±0.13</td>
<td>0.021</td>
</tr>
<tr>
<td>Retinol</td>
<td>-0.018±0.043</td>
<td>0.678</td>
<td>-0.045±0.083</td>
<td>0.590</td>
</tr>
<tr>
<td>( \alpha )-tocopherol</td>
<td>0.27±0.40</td>
<td>0.500</td>
<td>-0.080±0.49</td>
<td>0.870</td>
</tr>
<tr>
<td>( \gamma )-tocopherol</td>
<td>5.78±2.03</td>
<td>0.005</td>
<td>3.57±3.42</td>
<td>0.409</td>
</tr>
<tr>
<td>Vitamin E§</td>
<td>6.05±2.18</td>
<td>0.006</td>
<td>3.50±3.49</td>
<td>0.437</td>
</tr>
<tr>
<td>Copper</td>
<td>267.82±80.87</td>
<td>0.001</td>
<td>292.98±98.01</td>
<td>0.003</td>
</tr>
<tr>
<td>Iron</td>
<td>-77.48±99.58</td>
<td>0.437</td>
<td>61.86±134.98</td>
<td>0.647</td>
</tr>
<tr>
<td>Selenium</td>
<td>-4.50±2.74</td>
<td>0.102</td>
<td>-1.88±3.05</td>
<td>0.539</td>
</tr>
<tr>
<td>Zinc</td>
<td>29.64±27.56</td>
<td>0.283</td>
<td>40.38±37.25</td>
<td>0.280</td>
</tr>
</tbody>
</table>

\* \( \beta \)=Difference in analyte level for children severely anaemic at baseline compared to non-severely anaemic at baseline
† \( \alpha \)-carotene + \( \beta \)-carotene + \( \beta \)-cryptoxanthin; ¶ Lycopene + Lutein/Zeaxanthin; ‡ \( \alpha \)-carotene + \( \beta \)-carotene + \( \beta \)-cryptoxanthin + Lycopene + Lutein/Zeaxanthin; § \( \alpha \)-tocopherol + \( \gamma \)-tocopherol
levels of carotenoids seen in malaria (17). Plasma albumin and LDL cholesterol, for example, are lower in patients with falciparum malaria (44). Plasma albumin is a negative acute-phase protein (45,46) which decreases during malaria and also follows an increase in transcapillary escape rate (47). Das and colleagues observed that β-carotene and tocopherol were positively correlated with albumin and cholesterol (17), and capillary permeability, as measured by transcapillary albumin escape rate, urinary albumin excretion, and retinal fluoresce in angiography, is abnormal in about one-third of patients with uncomplicated falciparum malaria (48).

The significant differences in provitamin A carotenoid but not concentrations in non-provitamin A carotenoid on day 7 according to anaemia status may shed some light on the role of these nutrients during malaria infection. Anaemia in vitamin A-deficient humans can be reversed by giving β-carotene for vitamin repletion (40). Increased use of provitamin A carotenoids to synthesize retinol has been proposed by Thurnham and colleagues, who showed that the differences in concentrations of provitamin A carotenoids between malaria patients and controls were greater than the differences in non-provitamin A carotenoids (17). The finding that there was a significant difference in concentrations of provitamin A carotenoids between children with and without severe anaemia suggests that depleted levels of serum retinol are likely being compensated by increased use of provitamin A carotenoids. This is further supported by the finding that concentrations of provitamin A carotenoid in severely-anaemic children were not significantly different by day 7 compared to that at the start of the study.

While iron-supplementation trials in malaria-endemic areas have yielded conflicting results as to the effect of iron status on the incidence rates of malaria, parasitaemia, and prevalence of enlarged spleens, the beneficial effects of iron on haematologic status are clear. A recent Consensus Report of International Nutritional Anemia Consultative Group reviewed nine published and four unpublished randomized placebo-controlled iron-supplementation trials, of which eight showed a mean increase in concentration of haemoglobin between 0.2 and 1.2 g/dL, and three showed increases of over 2.5 g/dL (49). Menendez and colleagues reported that infants receiving iron supplementation had a lower frequency of severe anaemia compared to those who did not receive iron, yielding a protective efficacy of 28.8% [95% confidence interval 6.3-45.8] (50). Results of a meta-analysis of placebo-controlled trials of iron supplementation showed that the risk of anaemia was reduced by 50% (33).

Zinc, a requirement for normal immune function (13), is essential for the production of IgG, tumor necrosis factor-α, and interferon-γ, all of which are involved in resistance to malaria (33). During the acute-phase response, zinc is redistributed from plasma to lymphocytes and to the liver, causing decreased concentrations of zinc plasma and a microbiostatic environment (51). Results of a randomized placebo-controlled zinc-supplementation trial in Papua New Guinea showed that mean concentration of haemoglobin and the number of anaemic children did not differ among the two intervention groups at the end of the trial (22). Serum concentrations of zinc vary inversely with parasitaemia (13,52) and may preferentially protect against more severe malaria with high levels of parasitaemia (33). Increased use of zinc by parasites for the development of mature schizonts has been observed that eventually rupture the red blood cells they have infected (53). Lack of a significant difference in parasitaemia between the two groups of children may explain why the two groups did not have significantly different concentrations of zinc.

Besides iron and zinc, little is known about the role of trace elements, such as selenium and copper, during human malaria. In our study, the children who were severely anaemic at baseline had higher serum concentrations of copper than had those not severely anaemic. Serum concentrations of copper increase during the acute-phase response (54), and since the severely-anaemic children had a higher serum concentration of α1-acid glycoprotein, an acute-phase protein produced by hepatocytes, this may explain why being severely anaemic was associated with higher serum concentrations of copper. No studies with humans have examined the role of selenium, a component of the peroxide-decomposing enzyme glutathione peroxidase (55), during malaria. In our study, the severity of anaemia was not associated with serum concentrations of selenium.

One limitation of our study is that we could not assess the differences between the two groups of children regarding onset of illness and time elapsed before the children were brought to the clinic, since we did not have...
serial blood samples from the children. This may have affected differences in micronutrient levels between the severely- and the non-severely-anaemic children. In addition, follow-up was limited to 7 days, so micronutrient levels after malaria infection are not available. Because this study was clinic-based rather than community-based, an additional limitation was that concentrations of micronutrients in children prior to the malarial attack were not known.

In summary, our study has shown that the anaemic children with malaria had concentrations of retinol, carotenoid, and vitamin E and that severe anaemia was not significantly associated with different concentrations of micronutrients compared to non-severe anaemia. Our data corroborate the finding that increased use of provitamin A carotenoids occurs during malaria infection (17). While increased use of provitamin A carotenoids in children with malaria compared to controls has been previously reported, our study showed a difference in use of provitamin A carotenoids among children with differing severity of malarial anaemia. In addition, although concentrations of retinol are comparable in the two groups of children at baseline, children who are more anaemic may use more provitamin A carotenoids to meet the demand for retinol during an acute malaria attack. These observations suggest that the nutritional status is an important modulating factor in acute, uncomplicated malaria.

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