Plasma ferritin concentrations in anemic children: relative importance of malaria, riboflavin deficiency, and other infections

Delana A Adelekan and David I Thurnham

ABSTRACT  Anemia (hemoglobin < 110 g/L) was documented in 36 children of both sexes aged 1–12 y who were divided into two groups: malaria and other infections. The control subjects were 10 children of similar age with no anemia and without any apparent infections. Plasma ferritin concentrations (median, range) were higher in the anemic patients (203 µg/L, 21–5000 µg/L) than in control children (52 µg/L, 25–239 µg/L) although ferritin concentrations in those with malaria were still within the normal range (99 µg/L, 21–205 µg/L). In the rest of the anemic group, five patients had plasma ferritin concentrations > 1000 µg/L. There was no difference in riboflavin status between control subjects and patients or between the two anemic groups. Severity of anemia was no different between the two anemic groups either. The data indicate that riboflavin deficiency makes no contribution to the infection-induced elevation in plasma ferritin and that the contribution of malaria is smaller than that of other unidentified factors. Am J Clin Nutr 1990;51:453–6.

KEY WORDS  Anemia, malaria, infection, riboflavin deficiency, glutathione reductase activity, plasma ferritin

Introduction

Anemia is very prevalent in children in developing countries and iron deficiency is reported to be the most common cause of the anemia (1). Malaria is also an important cause of anemia in children in the tropics (2). Quite often, however, it is not possible to determine the cause of the anemia. In anemia of undetermined origin, chronic infection, helminthiasis, and malnutrition play important roles.

Several studies reported an increase in serum ferritin during malarial infection (3–5). Ferritin is an acute-phase reactant protein and its concentration in serum increases during acute infections or in aseptic inflammation (6–8). The anemia of malaria has been attributed to hemolysis and depressed marrow response, or dyserythropoiesis (3). There is evidence to suggest that damage to the liver and spleen by the malaria parasites may contribute to the elevated serum ferritin concentrations during malarial infections (5).

Riboflavin deficiency, like anemia, is very common in children in developing countries because of the generally poor state of nutrition (9). Riboflavin plays a role in the maintenance of the structural integrity of the red cell via the flavin-dependent erythrocyte glutathione reductase. Glutathione reductase is primarily responsible for the regeneration of reduced glutathione (GSH) from oxidized glutathione (GSSG) and for this function it requires a supply of flavin adenine dinucleotide (FAD), one of the metabolically active forms of riboflavin. GSH is a substrate for glutathione peroxidase that functions in the removal of hydrogen peroxide and other toxic lipid peroxides from the red cell, thereby protecting the red cell from oxidative damage (10, 11). Furthermore, glutathione itself acts as a scavenger of oxygen free radicals (12), which the malaria parasite and the cell-mediated immune response produce in large quantities. Riboflavin deficiency may therefore result in premature lysis of red cells and, like malaria, cause anemia.

This paper reports on the possible contribution of malaria, riboflavin deficiency, and other infections to anemia in children.

Methods

The study was conducted on 46 children aged 1–12 y of both sexes, presenting at the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria. The study was approved by the Ethical Committee of the Faculty of Health Sciences, Obafemi Awolowo University. Anemia was diagnosed in 36 patients on the basis of whole-blood hemoglobin concentration < 110 g/L (13), determined with a portable electronic hemoglobinometer, HemoCue (Leo Diagnostics AB, Helsingborg, Sweden). A detailed history was taken from each patient or the parent, with a view to determining the cause of the anemia.

On the basis of the history and further laboratory tests, the patients were divided into two groups according to the origin of the anemia as follows: group I—patients with malaria infec-

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Received March 9, 1988.
Accepted for publication April 12, 1989.
tion, diagnosed on the basis of fever on presentation and malaria parasites in peripheral-blood smears and/or response to chloroquine treatment, and group 2—patients with nonmalarial infection, such as upper respiratory tract infections, and hemoglobin concentration < 110 g/L. The 10 remaining children who were apparently well and with whole-blood hemoglobin concentration > 110 g/L served as the control group.

Venous blood from nonfasted patients and control subjects was collected in heparinized tubes. The tubes were centrifuged at 2000 × g for 10 min. Plasma was carefully removed and stored at −20 °C for later analyses. The buffy coat layer was discarded and red cells were washed three times in cold 0.15 mol saline/L. Washed red cells were lysed in cold distilled water to make a 1:2 hemolysate. The hemolysates were stored frozen at −20 °C for later analyses. Before analysis, the hemolysates were diluted 1:9 to make a final dilution of 1 in 20 and centrifuged to remove red cell ghosts.

Riboflavin status was measured on a Kone CD compact clinical analyzer (Kone Ltd, Calne, UK) as previously described (14). Riboflavin status was calculated as the ratio [the erythrocyte glutathione reductase activity coefficient (EGRAC)] of the maximal rates of change in absorbance per unit time in the presence and absence of FAD. EGRAC values > 1.30 are indicative of deficient status (15). Plasma iron concentration was measured on a Cobas centrifugal analyzer (Roche Products Ltd, Welwyn Garden City, Hertfordshire, UK) with the Roche iron ferrozine kit, and plasma ferritin was measured by radioimmunoassay by use of the Corning MAGIC ferritin kit (CIBA Corning Diagnostics Ltd, Halstead, Essex, UK).

Nonparametric analyses were used for comparisons between groups (Mann-Whitney U test). Associations between the hematological indices and riboflavin status are expressed by Pearson correlation coefficients (r).

Results

Values for the hematological and riboflavin status of anemic patients and control children are shown in Tables 1 and 2. There was no significant difference in the overall riboflavin status (EGRAC) of control children and that of all anemic children taken together (Table 1). Similarly, there was no significant difference between the median activities (basic and stimulated) of glutathione reductase in red cells and riboflavin status of patients in the two anemic groups (Table 2). Four patients in each anemic group were biochemically riboflavin deficient and 3 of the 10 control children were also deficient.

The hemoglobin concentration in the two patient groups taken together (80.2 ± 22.4 g/L, x ± SD) was significantly lower than the control value (120.5 ± 9.5 g/L; p < 0.025, t test). Patients with anemia and malaria had lower plasma ferritin concentrations than did patients with anemia of nonmalarial origin (p < 0.001, Mann-Whitney U test). Five of 19 (26%) patients with anemia of nonmalarial origin had plasma ferritin concentrations > 1000 μg/L. There was no significant difference between plasma ferritin concentrations of control children and of anemic patients with malaria. In the malaria group, there was no significant difference in the plasma ferritin concentration between patients deficient in riboflavin and those with normal riboflavin status. In general, however, all anemic patients taken together had an almost fourfold greater concentration of plasma ferritin in comparison with control children (p < 0.001, Mann-Whitney U test). Patients with anemia associated with malaria had significantly lower median and range iron concentrations than those in the other anemic groups (p < 0.001) and than control children (p < 0.003, Mann-Whitney U test).

To investigate the possible role of riboflavin status on the etiology of the anemia in the patients, the hematological variables were examined against the red cell glutathione reductase activities and the patients' overall riboflavin status (EGRAC). None of the hematological variables was significantly correlated with the activity of EGR with or without its cofactor, FAD, or with EGRAC in all anemic patients taken together. However, when patients were separated into groups according to the etiology of the anemia, significant correlations were recorded. In patients with malarial infection, plasma ferritin concentration was negatively correlated with basic (r = −0.53, p < 0.05) and stimulated (r = −0.55; p < 0.05) EGR activities in red cells. Similarly whole-blood hemoglobin concentration was negatively correlated with plasma ferritin concentration (Fig 1; r = −0.60, p < 0.05). Plasma iron was, however, positively correlated with basic (r = 0.64, p < 0.025) and stimulated (r = 0.58, p < 0.05) EGR activities in red cells of patients with malarial infection. In the malaria patients neither plasma iron nor plasma ferritin was significantly correlated with riboflavin status (EGRAC).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Hb</th>
<th>Basic EGR</th>
<th>Stimulated EGR</th>
<th>EGRAC</th>
<th>Serum iron</th>
<th>Plasma ferritin</th>
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<tbody>
<tr>
<td></td>
<td>g/L</td>
<td></td>
<td>μmol NADH·g Hb⁻¹·min⁻¹</td>
<td>μmol/L</td>
<td>μg/L</td>
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<tr>
<td>Median</td>
<td>119.0</td>
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<td>8.12</td>
<td>1.24</td>
<td>13.81</td>
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<td>Range</td>
<td>110–141</td>
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<td>6.28–11.22</td>
<td>0.98–1.58</td>
<td>2.74–21.61</td>
<td>25–239</td>
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<td>Anemic patients</td>
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<tr>
<td>Median</td>
<td>88.5</td>
<td>5.31</td>
<td>6.79</td>
<td>1.15</td>
<td>8.26</td>
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<tr>
<td>Range</td>
<td>20–109</td>
<td>2.23–16.69</td>
<td>2.63–18.41</td>
<td>0.99–1.77</td>
<td>1.82–41.97</td>
<td>21–5000</td>
</tr>
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<td></td>
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</table>

* Significantly different from median value of control subjects, p < 0.001 (Mann-Whitney U test).
Table 2
Erythrocyte glutathione reductase (EGR) activities and indices of iron status in anemic patients with and without malaria

<table>
<thead>
<tr>
<th></th>
<th>Hb</th>
<th>Basic EGR</th>
<th>Stimulated EGR</th>
<th>EGRAC</th>
<th>Serum iron</th>
<th>Plasma ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/L</td>
<td>μmol NADH·g Hb⁻¹·min⁻¹</td>
<td>μmol/L</td>
<td>μg/L</td>
<td></td>
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<tr>
<td>Median</td>
<td>96.0</td>
<td>5.23</td>
<td>6.49</td>
<td>1.15</td>
<td>5.12</td>
<td>99</td>
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<td>Range</td>
<td>57-109</td>
<td>3.02-16.69</td>
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<td>0.99-1.52</td>
<td>1.82-28.19</td>
<td>21-205</td>
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<td>Median</td>
<td>83.0</td>
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<td>7.89</td>
<td>1.15</td>
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<td>395*</td>
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<tr>
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<td>4.12-18.41</td>
<td>1.00-1.77</td>
<td>4.68-41.97</td>
<td>125-5000</td>
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<tr>
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</tbody>
</table>

* Significantly different from median value of control subjects, p < 0.001 (Mann-Whitney U test).

In patients with anemia of nonmalarial origin none of the hematological variables was significantly correlated with either of the EGR activities or with EGRAC.

Discussion

The pathological basis of the often life-threatening anemia of malarial infection is still not quite understood. The accompanying hemolysis, which is directly attributable to the malaria parasite, is often inadequate to explain the anemia. Another usual finding in malarial infection is an increase in serum ferritin concentration, partly as an acute-phase response and partly due to the hemolysis. In the present study, although the plasma ferritin values in the malaria patients are within the normal range, the significant negative correlation between whole-blood hemoglobin concentration (the index of anemia) and plasma ferritin concentration (Fig 1) in these patients points to a tendency for plasma ferritin concentration to increase during malarial infection. A common practice in this environment is for parents to administer antimalarial drugs to febrile children at home before bringing such children to the hospital. This reduces malaria parasitemia and may explain the somewhat normal plasma ferritin concentration in malaria patients in this study. The degree of the anemia (hemolysis) is directly related to the severity (parasitemia) of the malarial infection, and serum ferritin concentrations decline after antimalarial therapy (3).

The highest plasma ferritin values in the present study were recorded in patients with anemia of nonmalarial origin. Such high values are found in conditions of iron overload or in patients with acute or chronic liver damage (16). The plasma half-disappearance time for ferritin is very short, 10-15 min (17); therefore, any longstanding elevation may be due either to a continuous inflow, possibly by leakage from damaged liver cells, or to an augmented rate of ferritin synthesis (18). Malaria is endemic in this environment and children in the age group studied are the most vulnerable to malaria attack. Although the anemic patients had no malarial infection at presentation, it is conceivable that they had been exposed to repeated attacks of malaria in the past and the consequent liver damage by malaria parasites could account for the excessive amount of ferritin in their plasma. Furthermore, in anemia of undetermined origin, other chronic infections, helminthiasis, and malnutrition play important roles and may contribute to the elevated plasma ferritin seen in patients in this study.

A role for riboflavin in the pathogenesis of malarial infection has been established. Riboflavin deficiency was reported to be associated with low parasitemia in humans (19) and in experimental animals (20). In the present study the prevalence of riboflavin deficiency was no different in the malarial patients and the patients with anemia of nonmalarial origin. A recent study from India (21) showed that the degree of hemolysis in malarial patients is greater in those deficient in riboflavin than in those with normal riboflavin status. Serum ferritin concentration in malaria-infected and riboflavin-deficient patients would then be expected to be higher than that in malaria patients with normal riboflavin status. Our data did not bear this out because we were unable to demonstrate any significant difference in plasma ferritin concentration between malaria patients deficient in riboflavin and those with normal riboflavin status.
However, the severity of both the malarial infection and riboflavin deficiency in patients in this study is less than that in patients in the Indian study (21).

The results of the present study indicate that mild degrees of both malaria and riboflavin deficiency do not contribute appreciably to infection-induced elevation in plasma ferritin.

We acknowledge the assistance of the following people: Steve Smith of the Wolfson Research Centre, Birmingham, UK, for the serum ferritin measurement; John Smith of the Birmingham General Hospital, Birmingham, UK, for the plasma iron measurement; and the staff of the Children Emergency Ward, Obafemi Awolowo University Teaching Hospitals Complex, Ille-Ife Nigeria, for collecting the blood samples. We also thank Leo Diagnostics AB, Helsingborg, Sweden, for providing the HemoCue hemoglobinometer.

References