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EFFECT OF MALARIA ON SOLUBLE TRANSFERRIN RECEPTOR LEVELS IN TANZANIAN INFANTS


Abstract. The diagnosis of iron deficiency anemia in malaria endemic areas is complicated by the influence of the infection on the laboratory tests conventionally used to assess iron status. Determination of soluble transferrin receptor (sTfR) levels has been shown to be a sensitive indicator of iron deficiency in adults and is not affected by a range of infectious and inflammatory conditions. The utility of sTfR levels in the diagnosis of iron deficiency in malaria endemic areas remains unresolved.

Three hundred and fourteen infants in a rural area of southern Tanzania living under conditions of intense and perennial malaria transmission were studied to determine the utility of sTfR plasma levels in the assessment of iron deficiency anemia. Independent of the presence of anemia, malaria parasitemia was associated with a significant increase in sTfR plasma levels that were even higher than those found in iron deficiency anemia. We conclude that the measurement of sTfR levels does not have a role in the diagnosis of iron deficiency anemia in young children exposed to malaria infection.

INTRODUCTION

Iron deficiency is the most common cause of anemia and the most prevalent nutritional problem worldwide.1 Iron deficiency anemia is especially common in developing regions where infants, young children, and pregnant women are particularly at risk. Malaria infection is another major cause of anemia affecting the same populations. The geographic distribution of iron deficiency anemia and malaria transmission often overlap. Malaria infection affects the conventional laboratory tests of iron status such as plasma iron, ferritin, total iron binding capacity (TIBC) and transferrin saturation.2 Because of hemolysis, plasma iron may be also increased in malaria infection.3 Due to the complexity in the interpretation and the lack of specificity of the available tests for iron status determination, the clinical management of the anemic patient in malarious areas usually includes an empiric combination of treatments for anemia aimed at the most common causes. This influences the quality of health care as well as having important economic implications for the health sector. In the absence of definitive confirmation of iron deficiency through bone marrow examination, there is a need for non-invasive and specific tests especially in areas where iron deficiency is highly prevalent.

Together with ferritin concentration, soluble transferrin receptor (sTfR) serum levels have been suggested to be sensitive indicators of iron status in adults.4 Unlike other laboratory tests of iron status (such as plasma ferritin, iron, TIBC, and transferrin saturation), sTfR levels are not affected by infectious or inflammatory conditions, or pregnancy, in the absence of iron deficiency.5 In recent years the determination of sTfR levels has been proposed as a useful new tool in the diagnosis of iron deficiency in anemic patients.1 Serum levels of sTfR were increased in anemic adult patients with iron deficiency either with or without an associated inflammatory condition, while they were within the normal range in those with chronic infectious or inflammatory diseases alone.6

There have been very few reports on the usefulness of sTfR levels in the diagnosis of iron deficiency in children7 and only two from malaria endemic areas. These studies had contradictory results.8,9 One suggested that sTfR levels were not affected by malaria; the other observed that sTfR levels were significantly lower.8 Differences in the prevalence of iron deficiency and hemoglobinopathies, malaria endemicity and the age groups of the individuals studied were proposed to account for these discrepancies. Neither of these studies focused on infants, the group most affected by both iron deficiency and malaria anemia in highly endemic areas.10,11 We have therefore investigated the usefulness of sTfR levels in infants exposed to intense malaria transmission to assess iron deficiency anemia. These infants were originally enrolled into an intervention trial for the prevention of malaria and anemia.

MATERIALS AND METHODS

Study area and population. The study took place in the town of Ifakara, Kilombero district in southeastern Tanzania. The demographic characteristics and malaria epidemiology of the area have been described in detail elsewhere.12 In brief, malaria transmission is intense and perennial with an estimated entomological inoculation rate (EIR) of 300 infective bites per person per year in a nearby village.13 Clinical malaria is one of the main causes of hospital admission in children, the majority of cases and deaths occurring in individuals less than 1 year of age.14 Of the admitted children, 35% of those less than 5 years old and 41% of those less than 1 year of age had moderate or severe anemia (packed cell volume [PCV] < 25%) in infants; iron deficiency accounted for about 30% of such episodes.15

Study design and definitions. The current study was part of a randomized placebo-controlled trial of malaria chemoprophylaxis and iron supplementation for the prevention of
Soluble transferrin receptors and malaria

Malaria and anemia in infants. Children were randomized at two months of age to receive iron supplements, malarial chemoprophylaxis or placebo for 4 and 10 months respectively.\(^{14}\) Soluble transferrin receptor levels were measured in 314 randomly selected infants of the 611 (51%) who were visited at 8 months of age. At this visit, finger prick blood samples were collected for hematological, biochemical and parasitological analysis. Definitions used in the study are presented in Table 1.

Informed consent was obtained from all parents or guardians of the study infants. The study received ethical clearance from the Tanzanian Commission for Science and Technology and from the Ethical Committee of the Hospital Clinic in Barcelona.

**Laboratory procedures.** Blood samples were collected into EDTA and heparin microtainers (Becton Dickinson) and the separated plasma stored at \(-20^\circ\)C. Plasma soluble transferrin receptor levels were measured by an enzyme immunoassay using a kit from Orion Diagnostica (Espoo, Finland); according to the manufacturer the 25th and 97.5th percentiles of the reference distribution range for the kit were 1.3 and 3.3 mg/L respectively. Plasma ferritin was measured by a radioimmunoassay (Tandem-R Ferritin, Hybritech Inc, San Diego, CA); for the kit, the normal range for children was 10–83 ng/mL. Plasma iron (reference range 11–150 µg/dL) was measured colorimetrically using the ferrozine method without deproteination (Roche Diagnostics, Basel, Switzerland) in a Cobas Mira S analyzer. Plasma transferrin (reference range 208–347 mg/dL) and C-reactive protein were assayed by immunonephelometric methods (Dade Behring, Marburg, Germany).

Blood counts, hemoglobin (Hb), PCV, and the mean corpuscular volume (MCV) were determined on a semiautomatic cell counter (Sysmex F800 microcell counter, TOA Medical Electronics, Kobe, Japan).

Thick and thin blood films were stained and read for malaria parasites following standard, quality-controlled procedures.\(^{12}\) Hemoglobin electrophoresis was performed on cellulose acetate strips.

**Statistical methods.** Variables were compared between the anemia groups using regression analysis. The model was adjusted for the treatment groups. Spearman’s test was used for correlation analysis. Student’s \(t\)-test was used to compare MCV values. Logarithmic transformation was done for variables that were not normally distributed: PCV, ferritin, iron, transferrin concentration, sTfR, and C-reactive protein and parasite density. Statistical analysis was done using Stata Statistical Software version 5.0 (Stata Corporation, College Station, TX).

### TABLE 1

<table>
<thead>
<tr>
<th>Clinical malaria</th>
<th><em>Plasmodium falciparum</em> parasitemia of any density and axillary temperature (\geq 37.5^\circ)C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic malaria</td>
<td><em>P. falciparum</em> parasitemia of any density with an axillary temperature (&lt; 37.5^\circ)C</td>
</tr>
<tr>
<td>Iron deficiency</td>
<td><em>Plasma ferritin</em> &lt; 10 (ng/mL) and/or Plasma iron &lt; 11 (µg/dL) and Plasma transferrin concentration &gt; 347 (ng/dL)</td>
</tr>
<tr>
<td>Anemia</td>
<td>PCV &lt; 33%</td>
</tr>
<tr>
<td>Inflammation</td>
<td>C-reactive protein (CRP) &gt; 0.8 mg/dL</td>
</tr>
<tr>
<td>Microcytosis</td>
<td>Mean corpuscular volume (MCV) &lt; 70 fl</td>
</tr>
</tbody>
</table>

| Malaria parasitemia* | 48/314 (15.3) |
| Iron deficiency† | 37/314 (11.8) |
| Anemia (PCV < 33%) | 145/312 (46.5) |
| Inflammation (CRP > 0.8 mg/dL) | 98/305 (32.1) |
| Microcytosis (MCV < 70 fl) | 132/284 (46.5) |

* Anxial *Plasmodium falciparum*.
† Plasma ferritin < 10 (ng/mL) and/or Plasma iron < 11 (µg/dL) and Plasma transferrin concentration > 347 (mg/dL).

### TABLE 2

| Prevalence of hematologic and parasitologic variables in study children at 8 months of age |
|---------------------------------|---------------------------------|
| **Hb Genotype AS** | 39/125 (31.2) |
| **Malaria parasitemia** | 48/314 (15.3) |
| **Iron deficiency** | 37/314 (11.8) |
| **Anemia** (PCV < 33%) | 145/312 (46.5) |
| **Inflammation** (CRP > 0.8 mg/dL) | 98/305 (32.1) |
| **Microcytosis** (MCV < 70 fl) | 132/284 (46.5) |

RESULTS

Children were grouped according to the presence of anemia, iron deficiency, and malaria. Infants in group I (control group) did not have anemia, iron deficiency, or malaria (n = 142); group II were those with anemia alone (n = 86); group III had iron deficiency anemia (IDA) (but not malaria) (n = 25); and group IV had anemia plus malaria (but not iron deficiency) (n = 34). The remaining 27 infants in whom sTfR levels were measured belonged to two or more categories; that is, they were either anemic or not anemic with iron deficiency and malaria.

Hemoglobin genotype results were available in 125 children of whom 39 (31.2%) had the genotype AS (this prevalence is higher than that previously reported from the area\(^{14}\) and it is probably due to chance variation in the selection of the sample). Soluble transferrin receptor levels did not differ significantly by sex, presence of fever, microcytosis or Hb genotype (Menendez C and others, unpublished data). For this reason, analysis was done without distinction between these variables.

Hematological and parasitological features of the study population are shown in Table 2. The geometric mean sTfR level in the overall sample of children was 4.1 ng/ml (95%CI: 3.9–4.3). STfR levels correlated directly with transferrin concentration (\(r = 0.35; P < 0.001\)) and inversely with PCV (\(r = -0.40; P < 0.001\)) and iron (\(r = -0.10\), although the latter correlation was of borderline significance (\(P = 0.07\)). There was also a direct and significant correlation with the density of parasitemia (\(r = 0.36; P < 0.001\)). When compared with the control group, mean geometric sTfR levels were significantly higher in non-anemic children who had malaria infection (3.4 [95%CI: 3.2–3.6] versus 5.4 [95%CI: 3.9–7.6]; \(P < 0.001\)).

Hematological parameters (PCV and MCV), ferritin, iron, transferrin concentration, C-reactive protein, and sTfR were compared between the different categories of anemia using multiple regression analysis adjusting for the treatment group (Table 3). Mean sTfR concentrations were significantly lower in all intervention groups compared to the placebo group (Menendez C and others, data not shown). The analysis showed that both IDA and malaria-anemia were in-
Table 3

Hematologic and biochemical parameters in the study infants by anemia group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (Control)</th>
<th>Group II (Anemia alone)</th>
<th>Group III (IDA)</th>
<th>Group IV (Malarial-anemia)</th>
<th>Miscellaneous group</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)†</td>
<td>36.9</td>
<td>30.0***</td>
<td>29.9***</td>
<td>27.1***</td>
<td>36.4</td>
</tr>
<tr>
<td>(36.2, 37.6)</td>
<td>(29.4, 30.6)</td>
<td>(29.1, 30.7)</td>
<td>(25.8, 28.4)</td>
<td>(35.3, 37.5)</td>
<td></td>
</tr>
<tr>
<td>MCV (fL)‡</td>
<td>72.1</td>
<td>69.6*</td>
<td>65.4**</td>
<td>71.4</td>
<td>71.8</td>
</tr>
<tr>
<td>(6.7)</td>
<td>(6.6)</td>
<td>(7.6)</td>
<td>(7.1)</td>
<td>(7.4)</td>
<td></td>
</tr>
<tr>
<td>Plasma iron (µg/dL)†</td>
<td>37.9</td>
<td>35.2</td>
<td>23.9***</td>
<td>38.5</td>
<td>37.8</td>
</tr>
<tr>
<td>(35.0, 41.1)</td>
<td>(31.8, 39.1)</td>
<td>(18.3, 31.3)</td>
<td>(30.7, 48.3)</td>
<td>(30.1, 47.6)</td>
<td></td>
</tr>
<tr>
<td>Plasma ferritin (ng/mL)†</td>
<td>37.6</td>
<td>35.7</td>
<td>8.1***</td>
<td>62.7***</td>
<td>23.1*</td>
</tr>
<tr>
<td>(33.1, 42.8)</td>
<td>(29.6, 43.0)</td>
<td>(5.9, 11.2)</td>
<td>(43.6, 90.2)</td>
<td>(13.5, 39.7)</td>
<td></td>
</tr>
<tr>
<td>Plasma transferrin concentration (mg/dL)†</td>
<td>322.5</td>
<td>326.5</td>
<td>376.0***</td>
<td>355.0***</td>
<td>358.0***</td>
</tr>
<tr>
<td>(313.9, 331.3)</td>
<td>(316.2, 337.2)</td>
<td>(361.4, 391.3)</td>
<td>(336.4, 374.6)</td>
<td>(342.8, 373.7)</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)†</td>
<td>0.7</td>
<td>0.8</td>
<td>0.7</td>
<td>1.5***</td>
<td>0.8</td>
</tr>
<tr>
<td>(0.6, 0.8)</td>
<td>(0.7, 0.9)</td>
<td>(0.5, 1.0)</td>
<td>(1.0, 2.3)</td>
<td>(0.6, 1.1)</td>
<td></td>
</tr>
<tr>
<td>sTfR (ng/mL)†</td>
<td>3.4</td>
<td>4.2**</td>
<td>5.4***</td>
<td>6.8***</td>
<td>4.5**</td>
</tr>
<tr>
<td>(3.2, 3.6)</td>
<td>(3.8, 4.6)</td>
<td>(4.6, 6.3)</td>
<td>(5.8, 7.9)</td>
<td>(3.8, 5.4)</td>
<td></td>
</tr>
</tbody>
</table>

PCV = packed cell volume; MCV = mean corpuscular volume; sTfR = soluble transferrin receptor; anemia = PCV < 33%; IDA = iron deficiency anemia; miscellaneous group = anemic or non-anemic with iron deficiency and malaria.
* P < 0.05 versus controls adjusted for treatment group.
** P < 0.01 versus controls adjusted for treatment group.
*** P < 0.001 versus controls adjusted for treatment group.
† Data expressed as geometric mean (%95 confidence interval).
‡ Data expressed as arithmetic mean (SD).

Figure 1 shows the receiving operating characteristics (ROC) curves for sTfR in the identification of children with either malaria-related anemia or IDA. The measurement of sTfR levels gives a sensitivity and specificity greater than 80% in the identification of patients with malaria (A) while sensitivity and specificity could not be obtained simultaneously in the identification of patients with iron deficiency (B).

**FIGURE 1.** The receiving operating characteristics (ROC) curves for sTfR in the identification of children with either malaria-related anemia or IDA. The measurement of sTfR levels gives a sensitivity and specificity greater than 80% in the identification of patients with malaria (A) while sensitivity and specificity could not be obtained simultaneously in the identification of patients with iron deficiency (B).

Discussion

This study found that malaria infection was associated with a significant increase in sTfR plasma levels, even above those found in iron deficiency anemia and independent of a concomitant reduction in mean PCV level. This could be solely explained by the hemolysis associated with infection. The direct correlation found between sTfR levels and parasite density supports this. It could be argued that the high sTfR concentration associated with malaria infection could have been a misclassification of some iron deficient children in the absence of a gold standard test for iron deficiency.
such as bone marrow examination. However, a significantly 
(P < 0.001) lower MCV in the IDA group compared to the 
malaria-anemia group is consistent with the assumption that 
children were correctly classified in this study. It is unlikely 
that in clinical practice the diagnosis of iron deficiency ane-
mia would be more detailed than it has been in this study.

Plasma stTR levels are higher in conditions with high 
erythropoietic activity such as hemolytic conditions (includ-
ing hemoglobinopathies, congenital hemolytic anemias and 
traumatic hemolysis), iron deficiency, ineffective erythro-
poiesis and megaloblastic anemia.5,15 In one study, a sig-
nificant positive correlation was seen between stTR and 
erthropoietin levels in patients with IDA, suggesting that 
increased erythropoietin production could stimulate the syn-
thesis of stTR.

Our findings are in disagreement with two other studies 
carried out among malaria exposed individuals in which 
plasma stTR levels were either significantly decreased or not 
different in the presence of clinical malaria.8,9 Suppression 
of the bone marrow response to erythropoietin or its defi-
ciency were proposed to explain the decrease in plasma stTR 
levels in individuals with clinical malaria in Vanuatu.9 How-
ever, increased serum erythropoietin levels have been re-
ported in African children with severe malarial anemia16 and 
this may be in keeping with the finding of high stTR levels 
in association with malaria infection reported in our study. 
Erythrocyte progenitor cells are considered the main source 
of transferrin receptors in the body, and the fact that these 
receptors are increased in malaria may reflect an active ery-
throid marrow and would argue against the hypothesis that 
erthropoiesis is inhibited in malaria.19,20 Reticulocyte counts 
(which reflect the activity of the erythroid marrow) were 
found to be decreased in some studies but were normal or 
increased in others in association with malaria infection, sug-
gesturing furthermore that the erythropoietic activity in the 
bone marrow may be enhanced by malaria infection.21,22

Other reasons may explain the discrepancy between the 
other two studies in malaria endemic areas and ours regard-
ing stTR levels. First, in the current study, anemic patients 
were compared with a control group without anemia whereas 
in the other two studies the groups were not differentiated 
on the basis of anemia. Different malaria endemicity levels 
as well as differences in the age of the subjects studied may 
also contribute to these discrepancies. It is unlikely that the 
high prevalence of the Hb genotype AS in our study could 
account for the increased stTR levels in malaria-infected, 
anemic children, since stTR concentration was similar in Hb 
AA (geometric mean, 95% CI = 4.4 ng/ml, 3.9–4.8) and AS 
children (geometric mean, 95% CI = 3.8 ng/ml, 3.3– 
4.5). Furthermore, the proportion of children with the Hb 
genotype AS was lower among those with malarial-anemia 
than in the other two anemic and control groups (Menendez 
C and others, data not shown).

The presence of subpatent malaria parasitemia, or alter-
natively a malaria infection that has been recently resolved, 
may explain the moderate but significant increase in stTR 
levels in the group with anemia but without malaria or iron 
deficiency. It is known that the hemolysis of malaria infec-
tion may last for days or weeks after the parasitemia has 
been cleared.23 Therefore, the hematological effects of ma-
laria may remain, even though parasites are not detected on 
a blood smear examination.

This study has presented data showing that the measure-
ment of plasma stTR concentration does not have a role 
either in individual or population level assessment of iron 
deficiency in malaria endemic areas, at least in the age group 
with the highest prevalence of anemia and iron deficiency. 
Therefore, the accurate detection of iron deficiency anemia 
in malaria endemic regions by non-invasive methods re-
mains unresolved and more research is needed to find a di-
agnostic test which helps improving the clinical management 
of the anemic patients in these areas.

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