REDUCED SOLUBLE TRANSFERRIN RECEPTOR CONCENTRATIONS IN ACUTE MALARIA IN VANUATU

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Abstract. Soluble transferrin receptor (sTfR) concentration is a sensitive index of iron deficiency when used in conjunction with ferritin measurements in adults. One advantage of this assay is that unlike ferritin it does not appear to be affected by a range of infectious and inflammatory conditions or by pregnancy, rendering it a promising adjunct to the diagnosis of iron deficiency in tropical populations. We have measured plasma sTfR concentrations in a group of malaria patients (n = 21) and asymptomatic (18) and apasiticemic (76) controls in Vanuatu. Plasma sTfR concentration was significantly reduced in individuals with acute malaria (P = 0.003). While this observation provides evidence that erythropoietic suppression may be an important etiologic component in malarial anemia, it also suggests that malaria may be a confounding factor when interpreting sTfR concentrations in such populations. The role of sTfR in the diagnosis of iron deficiency in tropical populations remains to be established.

The diagnosis of iron deficiency can be difficult in tropical communities. Routine tests can be unreliable; for example, microcytosis is a poor indicator in populations where hemoglobinopathies are common and acute malaria can result in both anemia and elevated serum ferritin concentrations.

The transmembrane iron transport protein transferrin receptor (CD71) is a dimeric protein consisting of two identical 95-kD subunits. During red blood cell maturation, membrane transferrin receptor is degraded by endosomal digestion and an 85-kD fragment, soluble transferrin receptor (sTfR), is released into the circulation. Plasma levels can be measured using a sensitive ELISA method. It has been estimated that 80% of circulating sTfR originates from developing red blood cells. The STIR concentration is pathologically increased in the presence of tissue iron deficiency, and has been shown to be a sensitive index of this condition when interpreted in conjunction with ferritin measurements in adults. Unlike ferritin, sTfR is not affected by a range of infections, inflammatory conditions or by pregnancy and might thus be a useful tool in the assessment of iron status in tropical populations. We have investigated this possibility by measuring both sTfR and ferritin concentrations in malaria patients and control subjects living in Vanuatu.

MATERIALS AND METHODS

Subjects. The study was conducted on the Southwestern Pacific island of Santo, Vanuatu. Details of the population and the epidemiology of malaria have been published previously. Plasma samples were collected for the analysis of sTfR and ferritin concentrations from two sources: acute malaria samples were collected from consecutive patients presenting with clinical malaria to the Northern District Hospital in Luganville during the transmission season of 1994 and control samples were selected from a panel of plasma samples collected during the same malaria transmission season at cross-sectional surveys conducted in the communities served by the hospital. Two types of controls were selected: subjects with asymptomatic malaria and afebrile, individuals with no evidence of malaria parasitemia on microscopy. All subjects were assessed by a physician. Fever was defined as an axillary temperature > 37.4°C. Definitions for clinical malaria, derived as previously described, were as follows: Plasmodium falciparum and P. vivax were diagnosed in febrile subjects with > 500 and > 1,000 asexual parasites/µl of whole blood, respectively, who had no symptoms or clinical signs to suggest an alternative diagnosis. Asymptomatic malaria of both species was diagnosed in afebrile subjects with peripheral parasitemias of < 500/µl. Each case was matched to one or more controls of each category on the basis of age (date of birth within six months of the index), sex, and village of residence. At least one appropriate control of each category was available for each malaria case with the exception of three, for whom no samples were available from asymptomatic malaria controls fulfilling the matching criteria; however, multiple controls (maximum = 4) were available for 15 cases. Matching was conducted retrospectively by a clerical officer who was blind to hematologic, biochemical, and clinical details. Subjects with α-thalassemia were specifically excluded from the study since a significant increase in plasma sTfR concentration has been described in subjects with this condition. Ethical approval for this study was granted by the Scientific Ethical Research Committee, Department of Health (Port Vila, Vanuatu) and the Central Oxford Research Ethics Committee, (Oxford, United Kingdom). All subjects or their parents provided informed consent.

Sample processing. All blood samples were collected by venipuncture. Thick and thin blood films were made from fresh blood and aliquots preserved in both EDTA and heparin. Samples collected in the field were transported to the laboratory on melting ice and processed within 6 hr. Blood films were stained with Giemsa and examined for malaria parasites by standard methods. Blood counts were performed using samples preserved with EDTA on an automated cell counter (model CBCS; Coulter Electronics, Melbourne, Australia). All samples were then separated by centrifugation and plasma and pellet fractions were frozen at −20°C pending further processing in Oxford. Genotyping for α thalassemia was performed by Southern blotting.
transferrin receptor concentration was assayed by an ELISA (R & D Systems, Abingdon, United Kingdom) and ferritin was assayed by a radio-immune ELISA (Bio-Rad laboratories, Hemel Hempstead, United Kingdom) on freshly thawed samples. The normal ranges for these kits were 0.85–3.05 mg/L for sTfR and 14–200 μg/ml for ferritin. Statistical analysis was performed using STATA version 4.0 for Windows 3.1 (Timberlake, London, United Kingdom).

RESULTS

Twenty-eight malaria patients were originally recruited into the study; however, seven were found to have either heterozygous or homozygous α-thalassemia and were therefore dropped from the analysis. The final subject groups between groups analysis of variance [ANOVA] F[2, 820]=44,670 parasites/μL) and seventeen had P. falciparum infections (30,900 [13,200–72,450] parasites/μL). The asymptomatic malaria group included six subjects with P. vivax (295 [125–495]) and 12 with P. falciparum (100 [40–125]). Both sTfR and ferritin values were normalized by log transformation prior to analysis. Data from asymptomatic and symptomatic subjects were compared separately with controls using unpaired Student’s t-tests. Significant differences were seen between subject groups in log ferritin, hemoglobin, and log sTfR concentrations (Table 1): log ferritin concentration was higher (P < 0.0001) and hemoglobin and log sTfR concentrations were lower (P = 0.05 and P = 0.005, respectively) in clinical malaria patients than in controls. The data were further examined by regression analysis. Included in the model were the categorical variables age group (<2, 2–4, 5–9, 10–19, and >20 years), sex, and malaria status (clinical malaria, asymptomatic parasite positive and negative controls) and the continuous variable log ferritin. The only factors found to be significantly associated with sTfR concentration were clinical malaria infection (β [95% confidence interval] = −0.17 [-0.25 to −0.08], P < 0.001) and log ferritin (0.105 [0.04–0.17], P = 0.002). The only variable that was significantly different between asymptomatic subjects and controls was hemoglobin concentration (P = 0.008) (Table 1). Although the data also suggest that age has an influence on sTfR concentration, the numbers studied were too small to detect a significant trend (Table 2). Nevertheless, regression analysis did not suggest that age significantly confounded the between-group comparison.

DISCUSSION

Clinical malaria was associated with a significant decrease in plasma sTfR levels in Santo, Vanuatu. This observation suggests that erythropoietic activity is decreased during episodes of acute clinical malaria, supporting a role for erythropoietic depression in the pathogenesis of malarial anemia.14–17 This observation is consistent with studies that indicate that this may result either from acute erythropoietin deficiency18 or from the reversible suppression of the bone marrow response to erythropoietin.19,20 However, our findings do not agree with results from the only other study that has investigated sTfR levels in subjects with malaria.21 In that study, despite a more severely affected patient group (mean hemoglobin concentration = 6.1 g/dL), malaria was found to have no significant effect on plasma levels. This apparent discordance may result from a number of factors. First, it might be explained by differences in the prevalence of iron deficiency between the two areas. Whereas iron deficiency is rare in Vanuatu, affecting only 0.6–7.0% of the subjects in cross-sectional surveys,22 15–44% of the subjects in a study in Zaire were estimated to be deficient.23 Iron deficiency stimulates transferrin receptor synthesis and results in increased sTfR levels. Therefore, it is possible that a negative influence of malaria may have been masked by a positive influence of iron deficiency in Zaire. Second, important differences exist in both the epidemiology and pathogenic properties of malaria between Vanuatu and Afri-

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Asymptomatic malaria</th>
<th>Clinical malaria</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>76</td>
<td>18</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.6 (12.1)</td>
<td>9.4 (10.2)</td>
<td>11.9 (11.3)</td>
<td>F = 0.6, P = 0.6</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>12.3 (1.5)</td>
<td>11.9 (1.7)</td>
<td>10.7 (3.2)</td>
<td>F = 5.2, P = 0.007</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>79.4 (7.7)</td>
<td>79.2 (6.1)</td>
<td>79.0 (10.1)</td>
<td>F = 0.03, P = 0.97</td>
</tr>
<tr>
<td>Log₁₀ ferritin (µg/L)</td>
<td>1.60 (0.36)</td>
<td>1.70 (0.37)</td>
<td>2.08 (0.43)</td>
<td>F = 13.9, P &lt; 0.00001</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>39.4 (31.6–48.6)</td>
<td>49.8 (32.4–77.5)</td>
<td>120.8 (75.9–194.0)</td>
<td>P = 0.0001</td>
</tr>
<tr>
<td>Log₁₀ sTfR (mg/L)</td>
<td>0.39 (0.15)</td>
<td>0.40 (0.18)</td>
<td>0.23 (0.13)</td>
<td>F = 6.0, P = 0.003</td>
</tr>
<tr>
<td>sTfR (mg/L)</td>
<td>2.45 (2.3–2.7)</td>
<td>2.5 (2.0–3.1)</td>
<td>1.7 (1.2–2.3)</td>
<td></td>
</tr>
</tbody>
</table>

* ANOVA = analysis of variance; Hb = hemoglobin; MCV = mean cell volume; sTfR = soluble transferrin receptor. Values are the mean (SD).
† p < 0.05 versus controls, by two-tailed Student’s t-test.
‡ Values are the geometric mean (95% confidence interval).

### Table 2

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>n</th>
<th>Log₁₀ sTfR (mg/L)</th>
<th>SD</th>
<th>Geometric mean sTfR (mg/L)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>12</td>
<td>0.30</td>
<td>0.35</td>
<td>1.99 (1.35–2.88)</td>
<td></td>
</tr>
<tr>
<td>2–4</td>
<td>14</td>
<td>0.39</td>
<td>0.27</td>
<td>2.48 (2.38–3.34)</td>
<td></td>
</tr>
<tr>
<td>5–9</td>
<td>19</td>
<td>0.40</td>
<td>0.14</td>
<td>2.14 (2.19–2.83)</td>
<td></td>
</tr>
<tr>
<td>10–19</td>
<td>14</td>
<td>0.35</td>
<td>0.18</td>
<td>2.22 (1.81–2.72)</td>
<td></td>
</tr>
<tr>
<td>&gt;20</td>
<td>17</td>
<td>0.34</td>
<td>0.16</td>
<td>2.01 (1.70–2.41)</td>
<td></td>
</tr>
</tbody>
</table>

* Only clinically well, aparasitemic subjects were included in this analysis. Between group analysis of variance was not significant.
† CI = confidence interval.
ca.\textsuperscript{11,23,24} For example, whereas \textit{P. falciparum} is the major pathogen in Zaire, almost 40\% of the malaria seen in Vanuatu is caused by \textit{P. vivax}.\textsuperscript{11} Four subjects with this form of malaria were included in the current study whereas all subjects in the Zaire study were infected with \textit{P. falciparum}. While this particular factor does not appear to be responsible for the discrepancy (an analysis of the data excluding these patients did not materially affect the results), it is possible that the explanation may lie in other between-region differences in the host-parasite interaction. Third, unlike the current study, the one in Zaire took no account of the hemoglobinopathy status of subjects. It is now clear that the α-thalassemias are associated with increased sTfR concentrations, a phenomenon that probably reflects a mild degree of erythropoietic expansion in these conditions.\textsuperscript{12} Although no similar data are available for other hemoglobinopathies such as hemoglobin AS and heterozygous β thalassemia, it seems likely that they are also associated with increased sTfR concentrations, a phenomenon that could seriously confound the interpretation of sTfR measurements in tropical populations.

It is interesting to note that in the current study no significant differences were seen in hemoglobin, ferritin, or sTfR values between asymptomatic subjects and controls. This observation is particularly interesting in the light of previous work by Abdalla and others\textsuperscript{17} who observed that dyserythropoiesis was most common in patients with chronic malaria. In our study, in contrast to that of Abdalla and others, asymptomatic malaria patients were clinically well, essentially normal individuals with very low-grade parasitemia. Like others before us, we speculate that acute-phase reactants such as increased levels of tumor necrosis factor\textsuperscript{25,26} or inappropriately low levels of interleukin-10\textsuperscript{27} may contribute to the anemia of \textit{P. falciparum} malaria. Therefore, we suggest that the lack of evidence for such dyserythropoiesis in our asymptomatic group simply reflects their clinical state. On the other hand, we also acknowledge that the mean age of this group is lower than that of both the clinical malaria and control groups and that confounding by age might provide an alternative explanation.

In addition to the specific effect of malaria on sTfR concentrations, the current study also suggests that levels vary with age. Although too few subjects were included in the study to detect significant differences, a trend was shown towards increasing sTfR concentrations over the first 10 years of life followed by a secondary decrease. No published normal values are available for children; in their absence the value of sTfR measurements in the diagnosis of iron deficiency during childhood cannot be determined.

On the basis of the current study we conclude that in contrast to other infections,\textsuperscript{10} sTfR levels do not remain stable during episodes of clinical malaria. In addition, other factors may also lead to specific difficulties in the interpretation of sTfR concentrations in tropical populations including a high prevalence of hemoglobinopathies. Therefore, we suggest that measurement of sTfR can contribute little to the routine diagnosis of iron deficiency in such communities without considerable further work. In the absence of clinical malaria or other infectious or inflammatory processes, plasmat ferritin should remain the investigation of choice.

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REFERENCES

12. Srichaikul T, Wasansomsithi M, Poshyachinda V, Panikbutr N,


