Transferrin Saturation and Recovery From Coma in Cerebral Malaria

By Victor R. Gordeuk, Philip E. Thuma, Christine E. McLaren, Godfrey Biemba, Stenford Zulu, Anton A. Poltera, Jim E. Askin, and Gary M. Brittenham

To determine if the elevated transferrin saturations found in some patients with severe malaria are associated with an adverse outcome in cerebral malaria, we retrospectively measured baseline saturations in stored serum samples from 81 Zambian children with strictly defined cerebral malaria. The children had been treated with quinine, sulfadoxine-pyrimethamine, and intravenous infusions of either placebo (n = 39) or the iron chelator, desferrioxamine B (n = 42), in a previously reported trial (Gordeuk et al, N Engl J Med 327:1473, 1992). More than one-third of children in both the placebo- and iron chelator-treated groups had transferrin saturations exceeding 43%, which is 3 standard deviations above the expected mean for age. Among children receiving quinine and placebo, those with elevated transferrin saturations had a delayed estimated median time to recover full consciousness (68.2 hours) compared with those with saturations <43% (25.4 hours; P = .006). The addition of iron chelation to quinine therapy in children with high saturations appeared to hasten recovery (P = .046). We conclude that increased transferrin saturations may be associated with delayed recovery from coma during standard therapy for cerebral malaria and that serum iron and total iron binding capacity should be measured in future studies.

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CEREBRAL MALARIA is the most common clinical presentation and cause of death in severe malaria in many parts of the world. The condition is especially a problem among individuals with low prior immunity, such as young children in Africa. Cerebral malaria presents as an acute, diffuse symmetric encephalopathy in a patient with asexual forms of Plasmodium falciparum in the blood. Pathologically, sequestration of parasitized red cells (RBCs) in cerebral venules and capillaries is observed. As shown by electron microscopy, knobs on the membranes of infected RBCs mediate adhesion to vascular endothelial cells and contribute to the blockage of the microvasculature. Generalized cerebral edema, ring hemorrhages, and necrosis around cerebral veins are frequent postmortem findings, and elevated lactate concentrations in the cerebrospinal fluid indicate that cerebral hypoxia is present.

Studies in vitro and in vivo suggest that the final pathway in ischemic and hemorrhagic injury to the brain and other organs is mediated by iron-generated free radicals that induce lipid peroxidation damage to cellular and subcellular membranes. Although the inflammatory response to other severe infections is usually associated with cytokine-induced reductions in plasma iron levels, the presence of hemolysis and dyserythropoiesis that occur in children with severe malaria may raise transferrin saturation in some children with cerebral malaria. Elevations in transferrin saturation would diminish the capability of transferrin to complex with non–protein-bound iron that enters the circulation as a result of hemolysis or damage to tissues. Because free iron or hemoglobin may foster the production of free radicals or the formation of more toxic iron-centered activated oxygen species from radicals of lesser potency such as superoxide, elevated transferrin saturations could permit the generation of these toxic molecules and consequent tissue damage.

If free radical generation is important in the pathophysiology of cerebral malaria, more severe or prolonged central nervous system abnormalities might be expected in patients with elevated transferrin saturations. To determine how frequently elevated saturations develop in the setting of cerebral malaria and if they are associated with an adverse outcome, we retrospectively determined transferrin saturations at presentation in a cohort of children with cerebral malaria.

MATERIALS AND METHODS

Study population. The study population consisted of Zambian children who had been enrolled in a previously published prospec-
6 hours and medical care available in a rural African hospital was provided to all children. Of 83 children who were originally enrolled in the study, 81 had stored baseline serum samples available. These subjects serve as the basis of this report.

Serum iron studies. Baseline serum samples were stored at −20°C for 1 to 3 years before analysis. Iron and total iron binding capacity were determined by methods of the International Committee for Standardization in Hematology as modified for small volumes of serum. Transferrin saturation was calculated as follows: iron/total iron binding capacity × 100. The samples were not assayed for free hemoglobin, but visual evidence of sufficient hemolysis to substantially affect the serum iron measurement was not present. Serum ferritin was determined by a radioimmunometric assay (Ramco Diagnostics, Houston, TX).

Statistical analysis. Pretreatment clinical and demographic features were compared by Student’s t-test for continuous variables and by χ² test or Fisher’s exact test for proportions. Cox proportional-hazards models were used to examine the relation between baseline serum transferrin saturation and three measures of outcome: the time to recovery of full consciousness, the time to clearance of parasitemia, and mortality. As recommended by Pocock, a significance test for interaction between treatment with placebo or desferrioxamine B and baseline transferrin saturation was performed, followed by subgroup analyses if appropriate. Children were divided into two groups according to whether the baseline transferrin saturation was ≤43%, or within 3 standard deviations (SD) of the mean value for apparently healthy children. This reference value was taken from the second National Health and Nutrition Examination Survey (NHANES II) in the United States, in which African American children aged 3 to 4 years had a mean transferrin saturation of 22% ± 7%. Although reference values for transferrin saturation in Zambian children are not available, a survey conducted among 158 South African rural Black children 3 to 4 years of age in which the mean transferrin saturation was 21% supports the use of the NHANES II information.

The likelihood ratio test was used to compare the recovery from coma, clearance of parasitemia, and mortality between groups; all tests were two sided and a P value of less than .05 was considered to indicate significance. Because mean hemoglobin values were lower in subjects with elevated transferrin saturations (Table 1), we examined relationships between hemoglobin concentrations and transferrin saturations using correlation coefficients.

RESULTS

Eighty-one children were included in the study; 39 had received placebo and 42 desferrioxamine B in addition to

### Table 1. Clinical and Demographic Features According to Whether Subjects Received Placebo or Desferrioxamine B in Addition to Quinine and Sulfadoxine/Pyrimethamine

<table>
<thead>
<tr>
<th>Baseline Variables</th>
<th>Treatment With Quinine Plus Placebo (n = 39)</th>
<th>Treatment With Quinine Plus Desferrioxamine B (n = 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transferin Saturation ≤43% (n = 28)</td>
<td>Transferin Saturation &gt;43% (n = 14)</td>
</tr>
<tr>
<td>Females, no. (%)</td>
<td>11 (44)</td>
<td>8 (57.1)</td>
</tr>
<tr>
<td>Age, mo (mean ± SD)</td>
<td>33 ± 11</td>
<td>30 ± 8</td>
</tr>
<tr>
<td>Blantyre coma score (no. %)</td>
<td>4 (16.0)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>0</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>1</td>
<td>4 (16.0)</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>2</td>
<td>13 (52.0)</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>3</td>
<td>4 (16.0)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>4</td>
<td>4 (16.0)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>Duration of fever in hours before presentation (mean ± SD)</td>
<td>56 ± 34</td>
<td>60 ± 30</td>
</tr>
<tr>
<td>Duration of coma in hours before presentation (mean ± SD)</td>
<td>11 ± 12</td>
<td>16 ± 13</td>
</tr>
<tr>
<td>History of convulsions, no. (%)</td>
<td>19 (76.0)</td>
<td>12 (85.7)</td>
</tr>
<tr>
<td>History of treatment with chloroquine, no. (%)</td>
<td>20 (80.0)</td>
<td>77 (50.0)</td>
</tr>
<tr>
<td>History of treatment with traditional medicine, no. (%)</td>
<td>6 (24.0)</td>
<td>8 (57.1)</td>
</tr>
<tr>
<td>Transferrin saturation, % (mean ± SD)</td>
<td>23.2 ± 11.8</td>
<td>67.1 ± 21.6</td>
</tr>
<tr>
<td>Ferritin, μg/L (geometric mean and SD range)</td>
<td>358 (90-1,422)</td>
<td>560 (303-1,035)</td>
</tr>
<tr>
<td>Hemoglobin, g/dL (mean ± SD)</td>
<td>78 ± 1.7</td>
<td>5.9 ± 1.3</td>
</tr>
<tr>
<td>White blood cells, X10³/μL (mean ± SD)</td>
<td>11.0 ± 5.0</td>
<td>11.8 ± 5.3</td>
</tr>
<tr>
<td>Glucose, mg/dL (mean ± SD)</td>
<td>98 ± 54</td>
<td>84 ± 55</td>
</tr>
<tr>
<td>Peripheral blood asexual parasites, X10³/μL (geometric mean and SD range)</td>
<td>57.8 (6.7-500.2)</td>
<td>28.4 (1.8-443.9)</td>
</tr>
</tbody>
</table>

* N = 22. SD range is calculated as the antilog of the mean ± 1 SD. P values greater than .05 are rounded to the nearest one-tenth.
standard treatment with quinine and sulfadoxine/pyrimethamine. The two treatment groups were comparable at the time of enrollment with respect to the clinical and demographic characteristics summarized in Table 1. Thirty-three (41%) of 81 children enrolled in the study had transferrin saturations greater than 43% at presentation.

Effect of transferrin saturation on recovery from coma in all patients. Recovery of full consciousness during the 72-hour period of therapy with desferrioxamine B or placebo was analyzed in all 81 patients. Models adjusted for the coma score at presentation, the initial glucose concentration, the duration of coma before presentation, and the concentration of asexual parasites in the peripheral blood showed significant interaction between baseline transferrin saturation and treatment group ($P = .033$), providing evidence that the effect of treatment on recovery from coma was dependent on the initial level of transferrin saturation.

Transferrin saturation and recovery from coma in children receiving standard therapy plus placebo. Among the 39 children receiving placebo in addition to quinine and sulfadoxine/pyrimethamine, the level of transferrin saturation was found to be significantly associated with the rate of recovery of full consciousness after adjustment for the coma score at presentation and the initial glucose concentration ($P = .006$) (Fig 1). The rate of recovery of full consciousness in the group with transferrin saturations ≤43% (n = 25) was 3.3 times that in the group with saturations greater than 43% (n = 14; 95% confidence interval [CI], 1.2 to 9.0); the estimated median recovery times were 25.4 hours and 68.2 hours, respectively.

Transferrin saturation and recovery from coma in children receiving standard therapy plus the iron chelator, desferrioxamine B. Among the 42 children receiving the iron chelator, desferrioxamine B, in addition to quinine and sulfadoxine/pyrimethamine, transferrin saturation was not significantly associated with the rate of recovery of consciousness after adjustment for the duration of coma before presentation and the concentration of asexual parasites in the peripheral blood (Fig 2). Estimated median recovery times were 24.1 hours in 23 children with transferrin saturations ≤43% and 20.2 hours in 19 with saturations greater than 43% ($P = .4$).

Effect of iron chelation on recovery from coma in children according to transferrin saturation. In the 33 children with transferrin saturations greater than 43%, treatment with desferrioxamine B (n = 19) significantly influenced recovery of full consciousness after adjustment for coma score, peripheral blood parasite concentration, and glucose level at presentation ($P = .046$). The relative rate of recovery from coma in children receiving desferrioxamine B was 1.9 (95% CI, 0.9 to 10.4) times the rate in those receiving placebo. Among 48 children with transferrin saturations ≤43%, treatment with the iron chelator (n = 23) was not found to have a significant influence on recovery of full consciousness after adjustment for duration of coma before presentation ($P = .3$).

Parasite clearance. Parasite clearance was measured in 69 of the subjects. After adjustment for the log of the parasite concentration at time 0, no interaction was found between experimental treatment and baseline transferrin saturation in the group as a whole, and transferrin saturation did not have a significant effect on parasite clearance. Clearance of parasitemia was similar in children with transferrin saturations greater than 43% or ≤43% in both the placebo ($P = .7$) and the desferrioxamine ($P = .9$) treatment groups.

Mortality. Morality was 12.5% in 48 children with transferrin saturations ≤43% at presentation and 24.2% in 33 children with saturations greater than 43% at presentation. The joint effect of transferrin saturation and treatment with placebo or desferrioxamine B on mortality was evaluated in all 81 patients. Models adjusted for the coma score at presentation, the initial glucose concentration, duration of fever before presentation, and the concentration of asexual parasites in the peripheral blood did not show significant interaction between baseline transferrin saturation and treatment group ($P = .9$). Neither level of transferrin saturation ($P$...
= .2) nor experimental treatment \((P = .6)\) had a statistically significant effect on mortality.

**Relationships between transferrin saturations and hemoglobin concentrations.** Although hemoglobin concentration was not a significant baseline variable in the Cox proportional hazards modeling described above, a significant correlation was found between transferrin saturation and hemoglobin for all subjects \((r = -.49; P < .001)\). Thus, 24% of the variability in transferrin saturation could be explained by the variability in hemoglobin concentration. Similarly, significant correlations existed between transferrin saturation and hemoglobin concentration in children receiving standard therapy plus placebo \((r = -.64; P < .001)\) and in those receiving standard therapy plus iron chelation \((r = -.43; P < .005)\); respectively, 41% and 19% of the variability in transferrin saturation could be explained by the variability in hemoglobin concentration.

**DISCUSSION**

Although microvascular obstruction undoubtedly contributes to the pathogenesis of cerebral malaria,\(^1\) the exact mechanisms of coma in this condition remain undetermined.\(^24\) Some investigators have proposed a role for excessive effects of tumor necrosis factor and nitric oxide on the central nervous system,\(^25\) but direct evidence in support of this hypothesis has not been produced. We postulated that microvascular obstruction by *P. falciparum*-infected erythrocytes results in local ischemia and microhemorrhage, the release of free hemoglobin and iron, and the iron-dependent generation of free radicals that produce lipid peroxidant damage to cellular and subcellular membranes. We also postulated that elevated transferrin saturations that may occur in severe malaria might diminish the capacity to protect against peroxidant central nervous system damage and be associated with a delay in the clinical response to antimalarial therapy.

In this retrospective study, we found transferrin saturations more than 3 SD above the expected normal mean in 41% of 81 Zambian children who presented with cerebral malaria. Because both hemolysis and dyserythropoiesis are associated with elevated transferrin saturations,\(^14\) this finding in children with cerebral malaria would probably identify those with more severe sequestration of parasitized RBCs, hemolysis, bone marrow suppression, and tissue damage. The fact that mean hemoglobin concentrations were lower in children with elevated transferrin saturations is compatible with this possibility.

Our results indicate that, when other baseline variables are accounted for, elevated transferrin saturations are associated with delayed recovery of full consciousness among children with cerebral malaria receiving only standard antimalarial therapy with quinine and desferrioxamine B.\(^18\) The present study raises the possibility that therapy with desferrioxamine B in patients with cerebral malaria diminishes central nervous system damage by protecting against lipid peroxidation induced by iron-generated free radicals.

Mortality was lower in children with transferrin saturations in the normal range as compared with children with elevated saturations, but not significantly so. The sample size in this study may not have been sufficiently large to detect an effect of circulating iron levels on mortality in cerebral malaria, and this topic may warrant further study. The findings we report here are preliminary and should be confirmed in further prospective studies. Nevertheless, these results seem to warrant the conclusions that transferrin saturation may be a predictor of outcome in cerebral malaria and should be included as a baseline variable in future clinical studies.

**REFERENCES**

Transferrin saturation and recovery from coma in cerebral malaria

VR Gordeuk, PE Thuma, CE McLaren, G Biemba, S Zulu, AA Poltera, JE Askin and GM Brittenham