ASSOCIATION OF HELMINTH INFECTION WITH DECREASED RETICULOCYTE COUNTS AND HEMOGLOBIN CONCENTRATION IN THAI FALCIPARUM MALARIA

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Abstract. Following a study showing an association between Ascaris and protection from cerebral malaria, we conducted a cross-sectional study comparing admission hemoglobin concentrations in relation to exposure to helminth infection in 2 separate groups of patients: 111 cerebral malaria cases and 180 mild Plasmodium falciparum malaria cases. Hookworm infections were excluded. Mean hemoglobin concentrations were significantly lower in helminth-infected patients compared to those without helminths, both in the cerebral malaria group (10.1 ± 3 [n = 47] versus 11.2 ± 2.4 g/dl [n = 64], P = 0.04) and the mild malaria group (11 ± 2.5 [n = 89] vs 12.2 ± 2.7 g/dl [n = 91], P = 0.004). Median reticulocyte counts, only available in the cerebral malaria group, were lower in helminth-infected patients compared to those without helminths (15,340/23,760 per μl, P = 0.03). Adjustments for confounders such as body mass index did not alter these associations. These data are consistent with a mechanism causing anemia linked to differences in the immune response of helminth-infected patients during malaria.

INTRODUCTION

Malarial anemia is an important cause of morbidity and mortality.1 The pathophysiology of malarial anemia is still unclear but likely involves destruction of parasitized erythrocytes at schizont rupture, suppression of erythropoiesis by nitric oxide2 and cytokines such as TNF-α and IFN-γ,3 and destruction of non-parasitized red blood cells because of reduced red cell deformability.4 We have recently proposed to explain an association between Ascaris infection and protection from cerebral malaria by hypothesizing that endothelial and leukocyte CD23 ligation by IgE-anti IgE immune complexes induce nitric oxide, preventing cytoadherence of parasitized red blood cells.3 Although severe malarial anemia is rare in Thailand, our objective was to see if the magnitude of malarial anemia was influenced by helminth infection.

PATIENTS, MATERIALS, AND METHODS

We performed a retrospective study of 2 separate groups of malaria patients hospitalized between 1991 and 1997 at the Hospital for Tropical Diseases, Bangkok. First, we compared hemoglobin concentrations and reticulocyte counts on admission between helminth-infected and non-infected patients in a group of 111 cerebral malaria cases (reticulocyte counts on admission were only available for 61 cerebral malaria cases because they were part of routine clinical testing from 1991 to 1993). Second, we compared hemoglobin concentrations in relation to the presence or absence of helminths in a group of 180 mild P. falciparum malaria cases. The body mass index (BMI: weight/height²) and details of peripheral blood smears were available for 93 mild P. falciparum malaria patients. We did not compare mild malaria and cerebral malaria assuming they were different diseases.

Statistical methods. Quantitative values were compared using the ranksum test when distributions were non-Gaussian and the unpaired t-test for unequal variances was used for normally distributed variables. Adjustments for potential confounding variables were performed using multiple linear regression (STATA 6.0). Since helminths are related to socioeconomic factors that can potentially influence hemoglobin concentrations, we analyzed the relationship between helminth infection and hemoglobin in the Mon ethnic group alone. The Mon are political and economic refugees residing in the mountains of the western Thai border. Analyzing the relation between helminths and hemoglobin in this homogeneous group was assumed to avoid socioeconomic confounding.

All patients had a stool examination by direct examination of a wet preparation of 1.5 mg of stool. Since hookworm is known to be associated with iron deficiency we excluded patients with hookworm infection (135 patients). Since heavy Trichuris trichiura infections have been described as associated with blood loss,6 9 patients with infections of more than 5,000 eggs per gram of stool were excluded. For the subgroup where erythrocyte abnormalities were recorded, a logistic regression model was generated using the presence or absence of anisocytosis as the dependent variable.

RESULTS

There was no significant difference in the median duration of symptoms between helminth-infected and non-infected patients (respectively: median 4/4 days, quartiles 3/3 days and 6/7 days).

Types of helminths. There were 47 Ascaris lumbricoides infections, 61 moderate Trichuris trichiura infections, and 23 Strongyloides stercoralis infections. Forty-seven patients had single helminth infections, 30 had dual helminth infections, and 10 had 3 triple helminth infections.

Hemoglobin and helminths. In both study groups the mean hemoglobin concentration was significantly lower in helminth-infected patients when compared to patients without helminths (Table 1).

Adjustments for age, sex, ethnicity, mean corpuscular volume, P. falciparum parasitemia, and symptom duration did not alter the result (Table 1). There was a negative linear trend (P = 0.01) between the proportion of helminth-infected patients and the level of hemoglobin (Table 2). Similarly, there was a linear trend (P = 0.03) between the number of...
Table 1

Differences in mean hemoglobin concentration between helminth-infected and non-infected patients

<table>
<thead>
<tr>
<th>Helminth infections*</th>
<th>No helminth infection</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean hemoglobin concentration g/dL (SD) [number]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild malaria</td>
<td>11 ± (2.5) [89]</td>
<td>12.2 ± (2.7) [91]</td>
</tr>
<tr>
<td>Cerebral malaria</td>
<td>10.1 ± (3) [47]</td>
<td>11.2 ± (2.4) [64]</td>
</tr>
</tbody>
</table>

‡ Adjustments for age, sex, ethnicity, mean corpuscular volume, parasitemia, and symptom duration using multiple linear regression.

* Excluding hookworm infections.
† Student unpaired t-test

Anisocytosis and helminths. Anisocytosis was significantly more frequent in helminth-infected patients (crude odds ratio 2.9 [0.9–8.6]; P = 0.06). Using anisocytosis as a dependent variable in an unconditional logistic regression model, after adjusting for BMI and evolution duration, helminths were more frequently associated with anisocytosis (odds ratio 4.6 [1.3–16.3]; P = 0.02).

Reticulocytes and helminths. In the cerebral malaria group the median reticulocyte count was lower in helminth-infected patients (n = 24) than in patients without helminths (n = 37; 15,340 versus 23,760 per µL; 25% quartile 9,140—11,340 per µL; 75% quartile 27,180–45,000 per µL; P = 0.03). Adjustments for normalized reticulocyte counts stratified by age, sex, symptom duration, hemoglobin concentration, bilirubin level, parasitemia, mean corpuscular volume, and presence of renal failure did not alter the association (P = 0.003).

DISCUSSION

We have previously observed that helminth infection seems to confer a degree of protection against severe malaria. The present study suggests that helminth infection is associated with significantly lower hemoglobin concentrations and lower reticulocyte counts in patients with both mild and severe malaria. The relation between hemoglobin concentration and the development of cerebral malaria remains unclear.

The role of heavy Trichuris infections in iron deficiency and anemia has been reported. However, recent studies, one of them in Thailand,7 have not found an association with either Trichuris or Ascaris and anemia.6,9 Therefore, it seems unlikely that the observed difference in hemoglobin concentrations between helminth-infected and non-infected malaria patients was present before malaria occurred. Another possible explanation for these findings could be that the observed hematological difference was linked to preexisting anemia linked to undetected hookworm infections or nutritional abnormalities in helminth-infected patients. The in-

Table 2

Odds for helminth infections for different hemoglobin concentrations in patients with mild Plasmodium falciparum malaria

<table>
<thead>
<tr>
<th>Hemoglobin concentration</th>
<th>Mild malaria with helminth infection</th>
<th>Mild malaria with no helminth infection</th>
<th>Odds* (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6–8 g/dL</td>
<td>11</td>
<td>7</td>
<td>1.4 (0.6–4)</td>
</tr>
<tr>
<td>8.1–10 g/dL</td>
<td>21</td>
<td>13</td>
<td>1.6 (0.8–3.2)</td>
</tr>
<tr>
<td>10.1–12 g/dL</td>
<td>47</td>
<td>47</td>
<td>1 (0.7–1.5)</td>
</tr>
<tr>
<td>12.1–18 g/dL</td>
<td>10</td>
<td>24</td>
<td>0.4 (0.2–0.9)</td>
</tr>
</tbody>
</table>

* Score for linear trend, chi square 6.56 (1 df); P = 0.01.

Table 3

Odds of having hemoglobin concentration < 8 g/dL in relation to the number of different helminths infecting the patient

<table>
<thead>
<tr>
<th>Hemoglobin concentration</th>
<th>Mild malaria with hemoglobin &lt; 8 g/dL</th>
<th>Mild malaria with hemoglobin &gt; 8 g/dL</th>
<th>Odds* (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No helminths</td>
<td>5</td>
<td>87</td>
<td>0.06 (0.02–0.14)</td>
</tr>
<tr>
<td>1 helminth species</td>
<td>3</td>
<td>44</td>
<td>0.07 (0.02–0.2)</td>
</tr>
<tr>
<td>2 helminth species</td>
<td>5</td>
<td>25</td>
<td>0.2 (0.07–0.5)</td>
</tr>
<tr>
<td>3 helminth species</td>
<td>2</td>
<td>8</td>
<td>0.25 (0.05–1.2)</td>
</tr>
</tbody>
</table>

* Score for linear trend, chi square 4.81 (1 df); P = 0.03.
sufficient sensitivity of stool examinations could have missed the epidemiological link between hookworm and other helminths. However, adjusting for the body mass index and hypochromia did not alter these findings. Therefore, the differential presence of anemia in these patient groups does not seem to be explained by nutritional factors. It is possible that adjustments for mean corpuscular volume and hypochromia were not sufficient to un mask the confounding effect of iron deficiency, or that the statistical power of this study was limited in being able to detect a weak effect. Possibly, the hematologic stress resulting from malaria unmasked subclinical deficiencies in helminth-infected patients.

In accordance with our previous hypothesis and subsequent findings showing significantly higher concentrations of reactive nitrogen intermediates (RNI) in helminth-infected malaria patients (Nacher M and others, unpublished data), a possible explanation for the findings of the present study could be that helminth infections lead to decreased erythropoiesis through NO release. Since nitric oxide can reduce erythrocyte deformability, it could lead to increased red blood cell destruction. This hypothesis is supported by recent findings showing that RNI were negatively correlated with hemoglobin concentrations and were highest in young infants and after 5 years. Thus RNI could possibly contribute to the epidemiological patterns of severe anemia and cerebral malaria.

The role of the spleen in anemia in the patient populations studied here remain unclear. Previously, we have observed indirect signs of decreased clearance in helminth-infected malaria patients. One hypothesis could be that there is an adaptive, possibly NO-mediated reduction in splenic clearance avoiding selection of cytoadhesive strains. This would lead to a delay in crisis-related destruction of parasitized red blood cells and result in higher parasitemia and more profound anemia.

In conclusion, further studies are needed to elucidate the underlying mechanisms of the observations presented here. During malaria, preexisting helminth infections are associated with decreased hemoglobin concentrations and reticulocytosis and may aggravate the impact of malarial anemia. The fact that in patients without malaria the same helminths are exceptionally correlated with anemia suggests that the effect may be linked to the association of Plasmodium falciparum and helminths. Helminth-infected patients may be somewhat protected from cerebral malaria but more prone to severe anemia. Thus the prevalence of helminth infections and the intensity of malaria transmission could both provide partial explanations of the mutual exclusivity of severe malaria due to profound anemia versus cerebral complications.

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REFERENCES


