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.5 Although severe malarial anemia is rare in Thailand, our objective was to see if the magnitude of malarial anemia was influenced by helminth infection.

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 6/7days).

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, Plasmodium 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...
infecting helminths and patients with hemoglobin concentrations below 8 g/dL (P = 0.028) (Table 3). There was a negative correlation between the number of *Ascaris* eggs and hemoglobin (Spearman’s rho = −0.15, P = 0.045), between the number of *Trichuris* eggs and hemoglobin (Spearman’s rho = −0.13, P = 0.07), and between the number of *Strongyloides* larvae and hemoglobin (Spearman’s rho = −0.14, P = 0.06).

In the homogeneous Mon ethnic group, helminth-infected patients had lower mean hemoglobin concentrations (n = 61; 10.9 ± 2.5 g/dL) than those without helminths (n = 28; 12.4 ± 2.7 g/dL) (P = 0.009). This remained unchanged after adjusting for age, sex, fever duration, and mean corpuscular volume (P = 0.015).

**Other variables linked to anemia.** In the model used for adjustments in cerebral malaria (adjusted r-squared 0.45), there was a negative correlation between symptom duration and hemoglobin concentration (coefficient −3.17, P < 0.001). In the model used for adjustments in mild malaria (adjusted r-squared 0.24), there was a negative correlation between symptom duration and hemoglobin concentration (coefficient −2.21, P < 0.001), and a positive correlation between the mean corpuscular volume and hemoglobin concentration (coefficient 0.48, P = 0.02).

**Adjustments for nutritional status.** The association between helminths and low hemoglobin concentration remained significant (P = 0.006) when adding the BMI and presence/absence of hypochromasia as adjustment variables in the subgroup of mild malaria cases where anthropometric measurements and erythrocyte abnormalities were recorded (n = 93). Within this model (adjusted r-squared = 0.35), the symptom duration (coefficient −2.72, P < 0.001) was also negatively correlated with hemoglobin concentration whereas BMI was positively correlated with hemoglobin concentration (coefficient 20610, P = 0.03). The presence of hypochromasia was associated with low hemoglobin concentration (P = 0.01).

**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, have not found an association with either *Trichuris* or *Ascaris* and anemia. 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

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**Table 1**

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

<table>
<thead>
<tr>
<th>Helminth infection</th>
<th>Mean hemoglobin concentration (g/dL ± SD)</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild malaria</td>
<td>11 ± (2.5) [89]</td>
<td>0.004</td>
</tr>
<tr>
<td>Cerebral malaria</td>
<td>10.1 ± (3) [47]</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Excluding hookworm infections.
† Student unpaired t-test.
‡ Adjustments for age, sex, ethnicity, mean corpuscular volume, parasitemia, and symptom duration using multiple linear regression.

**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</td>
</tr>
<tr>
<td>8.1–10 g/dL</td>
<td>21</td>
<td>13</td>
<td>1.6</td>
</tr>
<tr>
<td>10.1–12 g/dL</td>
<td>47</td>
<td>47</td>
<td>1</td>
</tr>
<tr>
<td>12.1–18 g/dL</td>
<td>10</td>
<td>24</td>
<td>0.4</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>Helminth 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</td>
</tr>
<tr>
<td>1 helminth species</td>
<td>3</td>
<td>44</td>
<td>0.07</td>
</tr>
<tr>
<td>2 helminth species</td>
<td>5</td>
<td>25</td>
<td>0.2</td>
</tr>
<tr>
<td>3 helminth species</td>
<td>2</td>
<td>8</td>
<td>0.25</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 unmask 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,12,13 it could lead to increased red blood cell destruction.4 This hypothesis is supported by recent findings showing that RNI were negatively correlated with hemoglobin concentrations14 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.13

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.3 One hypothesis could be that there is an adaptive, possibly NO-mediated reduction in splenic clearance avoiding selection of cytoadhesive strains.15 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 helminth infections are exceptionally correlated with anemia suggests that the effect may be linked to the association of P. 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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