HEMOGLOBIN LEVELS RELATED TO DAYS OF ILLNESS, RACE, AND PLASMODIUM SPECIES IN COLOMBIAN PATIENTS WITH UNCOMPLICATED MALARIA

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Abstract. Prevalence of malaria-related anemia in disease-endemic regions of the American continents has been poorly studied. We describe the relationships between hemoglobin level and race, Plasmodium species, and days of illness in 150 Colombian patients with uncomplicated malaria diagnosed by thick blood smear. Hemoglobin was measured at admission and a standardized questionnaire was used to determine days of illness and other variables. Associations between hemoglobin and the variables were estimated and adjusted according to the other covariates using regression analysis. Plasmodium falciparum and P. vivax were found in similar proportions and mild anemia was present in 50% of the patients. Volunteers were classified as Afro-Colombians (61%) and non-Afro-Colombians (39%). An inverse relationship between hemoglobin and days of illness was identified, and a statistical interaction was found between race and P. falciparum infection in determining the hemoglobin concentration. These observations could guide the design of research to better understand malarial anemia.

INTRODUCTION

Malaria in humans is produced by four different Plasmodium species, of which Plasmodium falciparum and P. vivax are the most abundant and coexist in wide disease-endemic regions, particularly in Asia and the American continents. Anemia, one of the most common clinical manifestations of malaria, is responsible for great morbidity and mortality, particularly in malaria-endemic areas of Africa where P. falciparum is the predominant parasite species. Although anemia is well-documented in malaria-endemic regions of Africa and Asia, limited information is available about its prevalence in Latin America, where the predominant parasite species is P. vivax.

Malarial anemia is responsible not only for a significant proportion of child mortality worldwide, but also has a socioeconomic impact represented by school absenteeism, impairment in cognitive abilities, and neurologic sequelae. The mechanisms for anemia induction in patients with malaria are poorly understood, although it is accepted to be the result of a complex multifactorial process. Although parasite-induced hemolysis certainly contributes to the development of anemia, both a more general hemolytic process with destruction of non-parasitized erythrocytes and bone marrow impairment are considered major factors in inducing malaria-related anemia. Additionally, micro-nutrient deficiencies and coinfections have been described as contributing factors in malaria-endemic areas.

Based on the levels of parasitemia that patients with malaria develop, it has been estimated that lysis of infected erythrocytes, secondary to schizonts rupture, accounts for no more than 2–3% decrease in hematocrit level. However, it is well documented that survival of uninfected erythrocytes is significantly reduced. Although the causes of this decreased erythrocyte life span are not clear, increased hemolysis secondary to erythropagocytosis mediated by immunoglobulins and complement has been reported. Bone marrow impairment has also been described in patients with acute and chronic malarial infection. Erythropoiesis appears to be affected by unbalanced production of both pro-inflammatory and non-inflammatory substances such as interleukin-6 (IL-6), tumor necrosis factor-α, nitric oxide, IL-10, IL-12, and IL-18, whose production is usually stimulated by malarial infection. However, the role that the specific immune response to malaria plays in preventing or enhancing anemia is not well understood. Additionally, it is not clear if the intensity of malaria transmission by itself explains frequency of malaria complications. In Latin America, severe anemia and cerebral malaria are reported less frequently than in other continents. This might be explained by lower transmission intensity, parasite species, ethnic differences, or even by poor recording practices. Furthermore, the clinical determinants of malaria-associated anemia have not been reported in our population living in disease-endemic areas.

We report the results of a study to determine the relationships between hemoglobin levels and days of illness, race, and parasite species in patients with uncomplicated malaria from the malaria-endemic region on the Pacific coast of Colombia.

MATERIALS AND METHODS

Study population and design. A cross-sectional study was conducted between March and May 2004 among 150 individuals who asked for medical attention at the outpatient clinic of the Malaria Vaccine and Drug Development Center in Buenaventura, the main port on the Colombian Pacific coast. The area covered by the center is both urban and rural and includes an estimated 450,000 inhabitants. The region consists of a tropical rain forest with a mean temperature of 28°C, a relative humidity of 85%, and annual rainfall from 6,000 to 9,000 mm. Malaria transmission occurs throughout the year, with two small seasonal transmission peaks from April to May and from September to October. Although in most regions of Colombia P. vivax is prevalent, in this area, both P. vivax and P. falciparum are transmitted in approximately similar proportions. Communities in this region are racially mixed, with approximately 70% Afro-Colombians and 30% Spanish-
The prevalence of Fy-negative persons is approximately 52%.19

Selection criteria. Patients were asked to participate in the study if they were between 2 and 80 years old, had been diagnosed with malaria by a thick blood smear, and showed no clinical evidence of complicated infection. Volunteers were excluded if they reported any chronic disease or were pregnant. Ethical clearance to draw infected blood from human volunteers was obtained from the Institutional Review Board of Universidad del Valle. Written informed consent was also obtained from patients before blood was drawn or any additional information about them was collected. Parents or legal guardians provided consent for children less than 18 years of age.

Measurements. A questionnaire was used to obtain information about days of illness and other demographic and medical covariables, as shown in Table 1. This questionnaire was applied directly by two of the researchers (FZ and JV). Race was evaluated by direct observation of the participants, who were classified as either Afro-Colombian or non-Afro-Colombian.

Blood samples were obtained and the participants were immediately given curative doses of antimalarial drugs following the standard therapeutic protocol recommended by the Colombian Ministry of Social Protection. For blood collection, 3–5 mL of blood from each participant was drawn into an EDTA-Vacutainer® tube (Becton Dickinson, Franklin Lakes, NJ). Within one hour of the draws, hemoglobin levels were measured using a Coulter counter (ADVIA 60; Bayer, Tarrytown, NY). An automated hemoglobin measurement has a variation coefficient of 1–3%. Parasitemia was deter-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristics*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>Mean</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.8</td>
</tr>
<tr>
<td>Days of illness†</td>
<td>4.2</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.4</td>
</tr>
<tr>
<td>Parasitemia (%)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

† Reported time since the onset of any symptom by the patient.

In the second model, place of residence and parasite species were included. The third model was constructed by adding levels of parasitemia. This was considered important because the possible distorting effect of this covariate on the relationship between hemoglobin levels and days of illness could be strong. Finally, the fourth model included height as an anthropometrical variable. Height could not be considered a confounder in a classic way but was found to have an important effect on the estimates. An additional model was constructed with the variables that were independently associated with hemoglobin levels, including an interaction term between race and parasite species. Generally, the assumptions for multiple linear regression held for each of the models. Residual and influential analyses were performed, detecting one extreme observation, and analyses were done with and without this particular data, with similar results. All statistical analyses were performed using the statistical software package Stata version 7.0 (Stata Corporation, College Station, TX).

RESULTS

The statistical information for the variables studied in the patient population is summarized in Table 1. For most variables, complete data were available for most participants. The exceptions were days of illness, which were available for only 88.0% (132 of 150) of the participants, and Plasmodium species responsible for previous malaria episodes, which were available for 88.8% (95 of 107).

The mean age of participants, who were mostly male, was 28 years. Their median weight and height were lower than typical anthropometrical measurements for individuals of similar age and sex. Most participants (61%) were Afro-Colombian and most (57%) were from urban areas. In this group of patients, Afro-Colombians had approximately 1.8 kg/m² (95% confidence interval [CI] = −3.31 to −0.19) of body mass index less than non-Afro-Colombians adjusted by age, sex, and place of residence. The distributions of P. vivax and P. falciparum infections were almost equal, and only one patient had a P. malariae infection. The distribution of P. vivax infection in the participants was almost the same by race (21% in Afro-Colombians versus 29% in non-Afro-Colombians 29%). In contrast, the distribution of P. falciparum infection was more prevalent in Afro-Colombians
(41% in Afro-Colombians versus 9% in non-Afro-Colombians 9%). According to World Health Organization criteria, half of the participants had anemia but none was classified as severe. Seventy-five percent of the participants reported 2–5 days of illness before enrolling in the study, and approximately 70% reported having had at least one previous malarial episode. *Plasmodium falciparum* was the most frequently recalled infecting species. All participants reported symptoms of fever (100%), headache (99%), and diaphoresis (92%), whereas only 2% reported jaundice.

The mean hemoglobin level at admission was 12.4 g/dL (SD = 2.1). Spearman correlation coefficients between hemoglobin levels and days of illness, age, height, weight, parasitemia, and number of previous malarial episodes were −0.175 (*P* = 0.044), 0.447 (*P* < 0.001), 0.434 (*P* < 0.001), 0.359 (*P* < 0.001), −0.053 (*P* = 0.524), and 0.057 (*P* = 0.488), respectively. Cross-tabulations of crude and adjusted mean hemoglobin levels by days of illness, parasitemia, and previous malarial episodes are shown in Table 2. Mean hemoglobin levels decreased as days of illness increased, and this trend persisted after adjusting for potential confounders including age, sex, race, height, place of residence, smoking status, parasitemia, previous malarial episodes, and parasite species. No relationship was found between mean hemoglobin levels and either parasitemia quintiles or number of previous malarial episodes. As shown in Table 3, when mean hemoglobin levels of total number of *P. falciparum*– and *P. vivax*–infected patients were compared, no differences were observed (12.3 g/dL and 12.4 g/dL, respectively). However, when the levels of hemoglobin were discriminated by race and parasite species, non-Afro-Colombians infected with *P. falciparum* had a decrease of approximately 0.9 g/dL in hemoglobin levels compared with Afro-Colombians infected with the same parasite species. Hemoglobin levels in non-Afro-Colombians were 1.2 g/dL higher than in Afro-Colombians infected with *P. vivax*.

Table 4 shows the variables that were found to be independently associated with hemoglobin levels, including days of illness, race, and *P. falciparum* infection. For each day of illness reported, a mean adjusted decrease of 0.12 g/dL of hemoglobin occurred (95% CI = −0.22 to 0.02). Infection with *P. falciparum* was also found to be independently associated with hemoglobin levels (−1.49 g/dL, 95% CI = −2.55 to −0.44) when adjusted by race and when the interaction between race and parasite species was taken into account. As expected age, sex, and height were associated with hemoglobin levels.

### TABLE 2

Mean crude and adjusted hemoglobin (Hb) levels by days of illness, parasitemia, and previous episodes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean Hb (g/dL)</th>
<th>Adjusted mean for Hb (g/dL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Model 1</td>
</tr>
<tr>
<td>Days of illness†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–3</td>
<td>68</td>
<td>12.8</td>
</tr>
<tr>
<td>4–6</td>
<td>40</td>
<td>12.0</td>
</tr>
<tr>
<td>7–9</td>
<td>16</td>
<td>11.5</td>
</tr>
<tr>
<td>10–16</td>
<td>6</td>
<td>11.7</td>
</tr>
<tr>
<td><em>P</em>‡</td>
<td>0.037</td>
<td>0.010</td>
</tr>
<tr>
<td>Parasitemia (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(quintiles)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01–0.01</td>
<td>33</td>
<td>12.8</td>
</tr>
<tr>
<td>0.2</td>
<td>26</td>
<td>12.2</td>
</tr>
<tr>
<td>0.3</td>
<td>29</td>
<td>12.4</td>
</tr>
<tr>
<td>0.4</td>
<td>23</td>
<td>11.6</td>
</tr>
<tr>
<td>0.5–0.6</td>
<td>19</td>
<td>12.6</td>
</tr>
<tr>
<td><em>P</em>‡</td>
<td>0.310</td>
<td>0.597</td>
</tr>
<tr>
<td>Number of previous malarial episodes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(quartiles)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>38</td>
<td>12.4</td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>11.7</td>
</tr>
<tr>
<td>2–3</td>
<td>24</td>
<td>12.5</td>
</tr>
<tr>
<td>4–5</td>
<td>21</td>
<td>12.2</td>
</tr>
<tr>
<td>6–26</td>
<td>24</td>
<td>12.8</td>
</tr>
<tr>
<td><em>P</em>‡</td>
<td>0.401</td>
<td>0.727</td>
</tr>
</tbody>
</table>

*Model 1 = age, sex, race; Model 2 = age, sex, race, place of residence, and parasite species; Model 3 = age, sex, race, place of residence, parasite species, and parasitemia (or days of illness); Model 4 = age, sex, race, place of residence, parasite species, height and parasitemia (and/or number of previous episodes).*  
† Reported time since the onset of any symptom by the patient.  
‡ Estimated by analysis of variance and multiple linear regression t-test for the difference of the mean Hb between each one of the variables included.

### DISCUSSION

A linear and inverse relationship between self-reported days of illness and hemoglobin levels was found across uncomplicated malarial infection in the study population. We also found that hemoglobin levels were modified depending on race and parasite species. These findings are interesting because of the lack of information about malaria-related anemia in Latin America.

Hemoglobin levels were lower in non-Afro-Colombians infected with *P. falciparum* than in Afro-Colombians infected with the same parasite species. The differences in hemoglobin levels found between races in the group with *P. vivax* infections could also be explained by differences in body mass index. It is well known the direct relationship between hemoglobin levels and body mass. Moreover, the relationships between hemoglobin levels and days of illness, race, and parasite species were consistent in men and women and were independent of age, race, place of residence, height, smoking habit, and parasitemia.

The unequal distribution of parasite species by race suggests a selection bias. We expected to find approximately 70% of Afro-Colombians in both parasite groups according to the ethnic distribution in this area. The unexpected relationship of approximately 1:1 of *P. vivax* infections by race could be explained by the high prevalence of the Fy-negative phenotype in Afro-Colombians in this region. This selection bias would not change the relationships described in this report because there is no evidence that Fy status is associated with hemoglobin levels. Approximately 12% of the participants could not recall how long their illness had lasted, and this could introduce some selection bias in the regression coefficients estimates. However, we believe that this information was randomly missing and was not associated with hemoglobin levels, making it unlikely that it would explain the associations found. Misclassification bias due to the way days of illness were measured is possible but unlikely because of the acute nature of the illness and because most participants had previously had malaria and did not know their hemoglobin...
levels when the questionnaires were applied. Although physical differences between Afro-Colombians and non-Afro-Colombians are easily identified by observation, some misclassification bias may have occurred. Unknown confounders could explain some if not all of the described associations, but it is difficult to consider other specific variables in this case. Considering all of this, we believe that the associations we found are real and that no important bias was due to loss of information (selection), misclassification, or confounding.

In our study, we also showed that parasitemia was not associated with hemoglobin levels, which reinforces the idea that hemolysis of parasitized red blood cells does not explain anemia induced by malarial infection. Immunologic mechanisms have been proposed to explain malaria-related anemia, and evidence exists that acute hemolysis of non-infected erythrocytes, mediated by immunologic factors, plays a role.\textsuperscript{9,10} Despite this, most participants did not report jaundice at the time of diagnosis, which does not support the hypothesis that a hemolytic process caused hemoglobin levels to decrease over the span of the illness in the Colombian Pacific Coast population. We believe that increasing inflammation during disease progression could explain the association between hemoglobin levels and days of illness. Although the assessment of immunologic parameters was beyond the scope of this preliminary study, we speculate that inflammatory and immunologic responses to \textit{P. falciparum} infections are intrinsically different between racial groups. In a previous study of individuals infected with the Vietnam-Smith strain of \textit{P. falciparum}, blacks tolerated the disease better than whites.\textsuperscript{20}

The apparent susceptibility of non-Afro-Colombians to decreased hemoglobin levels during \textit{P. falciparum} infection is intriguing and not well understood. Since the non-Afro-Colombians in our study were living in the malaria-endemic area and no differences were found between Afro-Colombians and non-Afro-Colombians in number of previous malarial episodes or parasitemia levels, malaria exposure is likely to be similar in the two ethnic groups.

The observations described in this exploratory study could guide the design of research to better understand the mechanisms underlying the immunologic basis of malaria-related anemia and to compare the potential of both \textit{P. falciparum} and \textit{P. vivax} malaria to induce anemia.

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\begin{table}
\centering
\caption{Adjusted mean hemoglobin (Hb) values by race, parasite species, and tertiles of age*}
\begin{tabular}{|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Tertiles of age (years)} & \multicolumn{3}{c|}{\textbf{Plasmodium falciparum}} & \multicolumn{3}{c|}{\textbf{Plasmodium vivax}} \\
\hline & Non-Afro-Colombian & Afro-Colombian & Total & Non-Afro-Colombian & Afro-Colombian & Total \\
\hline
2–19 & 4 & 11.2 & 0.1 & 19 & 11.2 & 0.1 & 23 & 11.2 & 0.1 \\
20–34 & 4 & 11.3 & 0.1 & 16 & 12.7 & 0.5 & 20 & 12.4 & 0.9 \\
35–72 & 4 & 13.9 & 0.4 & 19 & 13.1 & 0.8 & 23 & 13.3 & 0.8 \\
Total & 12 & 12.1 & 0.2 & 54 & 12.3 & 0.1 & 66 & 12.3 & 0.1 \\
\hline
\end{tabular}
\begin{flushright}
\textsuperscript{*} Adjusted using multiple linear regression that includes the following covariates: age, sex, days of illness, parasitemia, previous malaria episodes, current smoker, and height.
\end{flushright}
\end{table}

\begin{table}
\centering
\caption{Variables independently associated with hemoglobin values*}
\begin{tabular}{|l|c|c|c|c|c|}
\hline
\textbf{Variables} & \textbf{Slope} & \textbf{95\% CI} & \textbf{t-test} & \textbf{P} \\
\hline
Days of illness\textsuperscript{†} & -0.12 & -0.22 & -0.02 & -2.40 & 0.018 \\
Afro-Colombian & -1.02 & -1.82 & -0.23 & -2.54 & 0.012 \\
\textit{Plasmodium falciparum} infection & -1.49 & -2.55 & -0.44 & -2.80 & 0.006 \\
Interaction term: & & & & \\
Afro-Colombian × & & & & \\
\textit{P. falciparum} & 1.94 & 0.67 & 3.22 & 3.02 & 0.003 \\
Age (per 5 years) & 0.15 & 0.04 & 0.25 & 2.77 & 0.006 \\
Sex (male) & 0.81 & 0.22 & 1.39 & 2.74 & 0.007 \\
Height (per 5 cm) & 0.17 & 0.09 & 0.25 & 4.36 & <0.001 \\
Constant & 6.58 & 4.21 & 8.94 & 5.50 & <0.001 \\
\hline
\end{tabular}
\begin{flushright}
\textsuperscript{*} Slope, \textit{P} values, and 95\% confidence interval (CI) were estimated by multiple linear regression in 130 observations. Slope is in g/dL.
\textsuperscript{†} Reported time since the onset of any symptom by the patient.
\end{flushright}
\end{table}


