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Malaria-Related Anemia in Patients from Unstable Transmission Areas in Colombia

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Abstract. Information about the prevalence of malarial anemia in areas of low-malaria transmission intensity, like Latin America, is scarce. To characterize the malaria-related anemia, we evaluated 929 malaria patients from three sites in Colombia during 2011–2013. *Plasmodium vivax* was found to be the most prevalent species in Tierralta (92%), whereas *P. falciparum* was predominant in Tumaco (84%) and Quibdó (70%). Although severe anemia (hemoglobin < 7 g/dL) was almost absent (0.3%), variable degrees of non-severe anemia were observed in 36.9% of patients. In Tierralta, hemoglobin levels were negatively associated with days of illness. Moreover, in Tierralta and Quibdó, the number of previous malaria episodes and hemoglobin levels were positively associated. Both *Plasmodium* species seem to have similar potential to induce malarial anemia with distinct cofactors at each endemic setting. The target age in these low-transmission settings seems shifting toward adolescents and young adults. In addition, previous malaria experience seems to induce protection against anemia development. Altogether, these data suggest that early diagnosis and prompt treatment are likely preventing more frequent and serious malaria-related anemia in Colombia.

INTRODUCTION

Although during the last decade, a significant decrease in the number of malaria cases has been reported worldwide, malaria still continues to be a major global public health problem.[1] Most studies on malaria-related anemia have been carried out in Africa, where *Plasmodium falciparum* is the predominant parasite species and children are the most vulnerable age group.[2,3] In contrast, limited data on malaria-related anemia are available from Latin America (LA) and the Caribbean endemic region.[2,4–7] The human population in this region has a different genetic background, *P. vivax* is the most prevalent malaria parasite species, malaria transmission intensity is low and unstable, and the young adult population seems to be at higher risk for malarial anemia than children.[8,9] As a consequence of this complexity, malaria and anemia exhibit epidemiological characteristics that seem to be unique to the region and remain poorly understood. In 2012, approximately 469,000 malaria cases (microscopically confirmed) were reported on the South American continent, mainly caused by *P. vivax* (approximately 74%) and *P. falciparum* (approximately 26%). Overall, this incidence corresponds to approximately 0.3% of the global malaria burden.[1]

The malaria transmission pattern in most endemic countries of the LA region is typically characterized as unstable (Annual Parasite Index [API] < 0.1 per 1,000 per year) for both *Plasmodium* species. However, in the Amazonas and on the Colombian Pacific Coast, it is considered stable (API ≥ 0.1 per 1,000 per year).[10,11] In 2012, three countries accounted for approximately 76% of cases: Brazil (52%), Colombia (13%), and Venezuela (11%).[1] Although a significant decrease in malaria morbidity and mortality has been reported in these two countries in recent years, little is known about the prevalence of anemia and other clinical manifestations. In Colombia, the total number of cases decreased from approximately 140,000 in 2000 to approximately 60,000 and 23 malaria-related deaths in 2012, corresponding to approximately 0.3 per 1,000 cases in this period.[9,12] This downward trend reflects the country's efforts toward timely diagnosis and effective treatment,[9] which may consequently modify the clinical profile of the disease.

Malaria clinical manifestations, including hematological changes, have been associated to multiple factors, such as demographic characteristics,[13] level of malaria endemicity,[14] nutritional status,[15,16] and malaria immunity[2,17]; thus, the pathophysiological process of malarial anemia seems to be especially multifactorial. Although malaria parasites induce hemolysis as the result of parasite replication and subsequent rupture of the host red blood cells (RBCs), other mechanisms, such as dysregulation and/or suppression of erythropoiesis, as well as direct and indirect destruction of non-parasitized RBCs (nRBCs) have been described.[2,3] Increased levels of nRBC apoptosis,[18] accelerated senescence,[19] and accelerated destruction of nRBCs by either opsonization or complement activation[20–22] have also been documented as major inducers of malaria-related anemia. Additionally, micronutrient deficiencies, including iron, folate, and vitamin B12, are known to significantly contribute to malaria-related anemia.[15,16] Furthermore, antimalarial drugs, such as primaquine, can trigger acute intravascular hemolysis in subjects with glucose-6-phosphate-dehydrogenase (G6PD) deficiency.[23] Moreover, it is known that individuals with malaria infection are frequently coinfected with intestinal helminthes.[24–26] However, the role of those coinfections in the epidemiology and clinical outcome of malaria-related anemia continues to be unclear.

The important role of malaria-related anemia in morbidity and mortality in regions with high endemicity has been extensively documented.[13–15,27–29] In contrast, limited information is available about the prevalence of malarial anemia in areas of low-malaria transmission intensity, like LA, where *P. vivax* is the predominant parasite species[30] and conditions for early diagnosis and prompt treatment are more favorable. Therefore, the purpose of this study was to determine the prevalence of malaria-related anemia and its relationships with RBC indices and independent variables in endemic areas of Colombia with different transmission intensities. Quantitative analysis was conducted in symptomatic patients with confirmed malaria.

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MATERIALS AND METHODS

Study population. In total, 929 symptomatic malarial patients with monoinfection by *P. falciparum* or *P. vivax* were enrolled as part of a study to determine the clinical profile of malaria in Colombia. The study was carried out between 2011 and 2013 in three malaria-endemic sites selected based on distinct transmission patterns and sociodemographic characteristics. After microscopic malaria diagnosis, patients 3–87 years of age received oral and written information about the study, and after voluntarily agreeing to participate as research subjects, each signed the informed consent (IC) form previously approved by the Institutional Review Board (IRB) of the Malaria Vaccine and Drug Development Center (MVDC). After the IC was obtained from each adult individual and a signed informed assent was obtained from patients 7–17 years of age and their parents/guardians, a trained member of the study staff collected clinical, demographic, and epidemiological information from each participant using a standardized questionnaire. Previous malaria episodes were self-reported and not confirmed by laboratory records. The local health provider treated all individuals as soon as the blood sample was drawn using the national protocol for malaria treatment.[31] Patients infected with *P. vivax* were treated orally with curative doses of chloroquine (25 mg/kg provided in three doses) and primaquine (0.5 mg/kg daily for 14 days). Patients infected with *P. falciparum* received artemether plus lumefantrine (total of six doses over 3 days). Each individual received a unique code number to simplify data collection and identification.

Sites of study. Three malaria-endemic settings with different transmission patterns were selected: Tierralta (Department of Córdoba) in the northern region of Colombia, Quibdó (Department of Chocó) in the western region, and Tumaco (Department of Nariño) in the southwest region along the Pacific coast. Both *P. vivax* and *P. falciparum* are transmitted in these regions in different proportions, with an unstable endemic pattern[8,12] (Figure 1). Tierralta has a population of approximately 90,000 inhabitants, with most inhabitants characterized as mestizos and an indigenous minority (Emberá Katío); the predominant malaria parasite species in this region is *P. vivax* (83%). Tumaco, situated close to the border with Ecuador on the Pacific coast of Colombia, has a population of 161,490 inhabitants who are predominantly Afro-descendants, and there is an indigenous minority (Awá); the predominant malaria *Plasmodium* species in this region is *P. falciparum* (79%). Quibdó, located in the northwestern region of Colombia near the border with Panama, has a population of approximately 100,000 inhabitants who are mainly Afro-descendants and Afro-Amerindians; most malaria cases (approximately 60%) are caused by *P. falciparum.* According to the National Surveillance Service of Colombia (SIVIGILA), the highest incidence of malaria occurs mainly in young adults (16–30 years of age) followed by children (5–15 years of age).[9]

Laboratory tests. Capillary blood samples collected by finger prick were used for microscopic malaria diagnosis and parasite density counts. Giemsa-stained thick blood smears were used to determine the number of parasites per microliter (parasitemia) by counting the number of parasites per 200 white blood cells (WBCs), and they were normalized using the actual WBC counts. Microscopic diagnosis in all participants was confirmed by real-time quantitative polymerase chain reaction (RT-qPCR).[32] Venous blood (7–15 mL) was collected by venipuncture into Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ) with ethylenediaminetetraacetic acid (EDTA) and used for complete blood cell counts by an automated hematology analyzer (KX-21N; Sysmex, Kobe, Kansai, Japan) at each local healthcare facility. The following parameters were measured: hemoglobin (Hb), hematocrit (Htc), red cell distribution width (RDW), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), and RBC count. Hb concentrations were used for diagnosing anemia according to the World Health Organization (WHO) criteria adjusted by age and gender.[33] Severe anemia was classified as Hb level < 7 g/dL following the Colombian guidelines for malaria.[31]

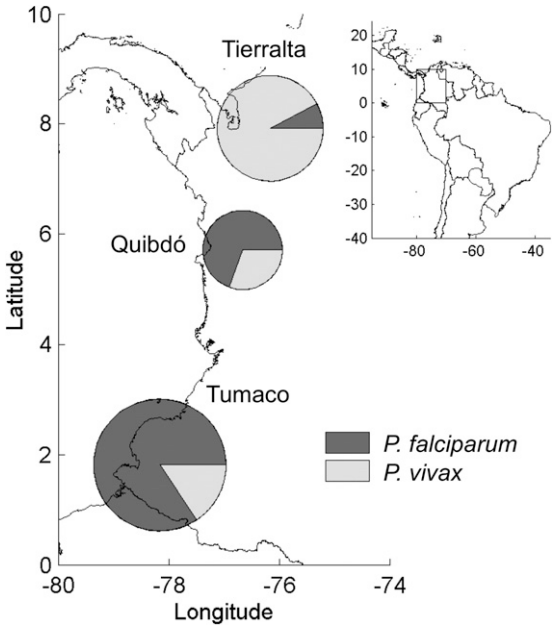

FIGURE 1. *Plasmodium* distribution cases in the study areas. The three study sites of Tierralta (Department of Córdoba), Tumaco (Department of Nariño), and Quibdó (Department of Chocó) are shown. The pie charts display the proportions of *Plasmodium* species per site.

Statistical analysis. Study data were collected and managed using REDCap.[34] All variables were analyzed using descriptive statistics. Mann–Whitney U and Kruskal–Wallis tests were used to compare medians among two or more groups, respectively. Dunn's multiple comparison test was used for post-hoc analysis. Spearman's rank correlation (r_s) was used to determine the correlation between continuous variables. χ^2 tests and Fisher's exact tests were used to compare proportion differences. Associations between Hb levels and other continuous variables were adjusted using linear regression. Multiple regression models were constructed to evaluate the effect of different groups of potential confounders on the relationship between Hb levels and relevant independent factors. Models used for analysis included model 1 (adjusted by age, gender, race, and species), model 2 (model 1 plus days of illness), and model 3 (model 2 plus previous malaria episodes, number of episodes, and parasitemia). A P value < 0.05 was considered statistically significant. Statistical analysis was performed using MATLAB 2013a. (The MathWorks, Inc., Natick, MA). A MATLAB script was used to generate the results presented.

RESULTS

Population characteristics. In total, 929 subjects with similar gender distribution (54.8% male versus 45.0% female) were enrolled in the study. Median age of patients in Quibdó and Tumaco was similar (23 and 24 years old, respectively) and higher than in Tierralta (18 years old) (Table 1). Most participants in Tierralta were mestizo" (73.3%), whereas those in Tumaco and Quibdó were mainly Afro-descendants (82.8% and 74.6%, respectively) (Table 1). A total of 48.3% of all patients reported previous malaria episodes, with a mean of 1.8 episodes (median = 0; 95% confidence interval [95% CI] = 1.6–2.0). A higher percentage of patients from Tierralta (71.9%) self-reported previous malaria episodes than those from Tumaco and Quibdó (30.1% and 47.8%, respectively; $P < 0.0001$, χ^2 test) (Table 1). Likewise, the number of previous episodes reported in Tierralta ranged between 1 and 25 and was significantly different than the other two sites, with 45.0% of patients reporting ≥ 4 previous episodes. The majority of patients in Tumaco (90.0%) and Quibdó (69.7%) reported < 3 episodes, with ranges between 1 and 7 and 1 and 10 episodes, respectively. In general, patients attended the healthcare facilities promptly, which are all located by road within 1 hour from their households, for malaria diagnosis (mean = 5.4 days; median = 4 days; interquartile range [IQR] = 2–6 days). However, the disease duration before consultation was significantly different in the three study sites (Table 1). Few patients reported 15–55 days of illness: 1.1% of patients in Tierralta, 3.4% of patients in Tumaco, and 8.0% of patients in Quibdó (data not shown).

Only patients with *Plasmodium* monoinfection were enrolled. Parasite species distribution was statistically different in the three study sites; however, the overall distribution indicated a total of 441 (47.5%) participants presenting with *P. vivax* malaria and a total of 488 (52.5%) participants with *P. falciparum* infection (Figure 1). Infection with *P. falciparum* was more prevalent in Tumaco (84.4%) and Quibdó (67.4%), particularly in Afro-descendants, whereas *P. vivax* was predominant in Tierralta (92.1%), with a similar proportion of cases in both non–Afro- and Afro-descendants populations (53 of 58 and 275 of 298, respectively; data not shown). The geometric mean parasitemia was different among study sites as shown in Table 1 ($P < 0.0001$; Kruskal–Wallis test). The majority of patients (approximately 96%) had low to moderate parasitemia (< 20,000 parasites/μL), with significantly higher geometric mean parasitemia in patients with *P. vivax* infection than those with *P. falciparum* infection (2,939 versus 1,590 parasites/μL; $P < 0.0001$, Mann–Whitey test). A limited number of patients (3 of 929) presented with hyperparasitemia (> 50,000 parasites/μL)[31]: two patients with *P. falciparum* (Quibdó) and one patient with *P. vivax* (Tumaco; data not shown).

Hb levels are associated with days of illness and the number of previous malaria episodes. Malaria-related anemia, defined as described before, was observed in 36.9% of patients, with quite similar percentages observed among study sites. Severe anemia (Hb < 7 g/dL) was found in approximately 0.3% of patients: one *P. falciparum* case in Quibdó and two *P. vivax* cases in Tierralta (Table 2). In Tumaco, mild anemia was 1.4 times more frequently caused by *P. vivax* malaria than *P. falciparum* ($P = 0.018$), whereas a similar percentage of anemia between species was observed in Tierralta and Quibdó.

Mean Hb levels were observed within the normal range (11–16.5 g/dL) in the three study sites; however, values found in Tierralta were greater and showed significant differences ($P < 0.0001$) with respect to the other two sites (Table 1). The mean Hb level in females was significantly lower than that found in males (11.7 versus 13.2 g/dL; $P < 0.0001$), but both were close to the normal range. Hb levels increased significantly with age in all study sites. Individuals with anemia were younger (median age = 17 years; IQR = 11–34 years) than non-anemic individuals (median age = 22 years; IQR = 16–37 years; $P < 0.0001$). Subjects from Tierralta and Quibdó reporting no malaria episodes during their lifetime had significantly lower Hb levels during the current malaria episode than those with high numbers of malaria episodes (Figure 2).

TABLE 1
Characteristics of malaria patients

	Tierralta ($N = 356$)		Tumaco ($N = 435$)		Quibdó ($N = 138$)		
	Median	IQR	Median	IQR	Median	IQR	P value*
Age (years)	18†	12–33	24	16–38	23	14–41	< 0.0001
Number of previous malaria episodes	2	0–5	0	0–1	1.0	0–2	< 0.0001
Days of illness	4.0	2–6	4.0	2–7	6.6	3–8	0.001
Parasites per microliter‡	2,753	1,326–6,007	1,393	594–3,450	4,198	1,827–9,451	< 0.0001
Hb (g/dL)	12.9†	11.6–14.5	12.3	11.2–13.5	12.1	10.8–13.4	< 0.0001
Hematocrit (%)	38.6†	34.7–42.6	36.9	33.4–40.7	35.9	32.4–40.2	< 0.0001
RDW (%)	10.5†	8.3–12.0	13.9	13.0–14.5	13.1	11.9–13.8	< 0.0001
MCV (fL)	78.4	76–81	84.2	79–90	83.2	80–88.0	< 0.0001
MCH (pg)	26.4	24.8–28.1	28.1	26.7–29.8	28.1	26.5–29.8	< 0.0001
MCHC (g/dL)	33.4	32.1–34.4	33.5	32.1–34.5	33.6	32.8–34.7	0.064
RBC ($\times 10^6$/μL)	4.9†	4.5–5.3	4.4	4.0–4.8	4.3	3.8–4.8	< 0.0001
Frequencies	*n*	Percent	*n*	Percent	*n*	Percent	P value§
Male	213	59.8	216	49.7	80	58.0	0.012
Race							< 0.0001
Afro-descendant	58	16.3	360	82.8	103	74.6	
Mestizo	261	73.3	43	9.9	12	8.7	
Indigenous	24	6.7	13	3.0	8	5.8	
White	10	2.8	15	3.4	13	9.4	
Afro-Amerindians	3	0.9	4	0.9	2	1.4	

* P value using Kruskal–Wallis test.
† Statistically significant: Tierralta versus Tumaco or Quibdó. Dunn's multiple comparison test.
‡ Geometric mean (95% CI).
§ P value using χ^2 test.

TABLE 2
Anemia distribution per parasite species and study site[33]

Classification	Tierralta ($N = 356$) n (%)		Tumaco ($N = 435$*) n (%)		Quibdó ($N = 138$) n (%)	
	P. falciparum	*P. vivax*	*P. falciparum*	*P. vivax*	*P. falciparum*	*P. vivax*
Non-anemia	20 (71)	234 (71)	224 (61)	31 (46)	53 (55)	24 (57)
Mild to moderate anemia†	8 (29)	94 (29)	143 (39)	37 (54)‡	43 (45)	18 (43)
Severe anemia§	0	2 (0.6)	0	0	1 (0.7)	0

*$P = 0.018$ using χ^2 test (*P. falciparum* versus *P. vivax*).
†Adjusted by age and gender.
‡Most frequent data.
§Severe anemia was defined as Hb levels < 7 g/dL.

A significant negative correlation between anemia and days of illness was observed in Tierralta only ($r_s = -0.172$; $P = 0.013$).

To analyze the independent contribution of potential confounders on Hb levels, we constructed a series of multivariate regression models with patients from each site. In unadjusted analyses, age, gender (male), race (Afro-descendants), parasite (*P. falciparum*), days of illness, previous malaria episodes (yes), number of previous malaria episodes, and parasitemia were independently associated with Hb levels. After adjusting, age and gender (male) were significantly associated with Hb levels at all sites (Tables 3–5).

In Tierralta, Hb levels increased with a greater number of previous malaria episodes but decreased with longer duration of illness, even after adjusting for potential confounders, including age, gender, race, parasite species, previous malaria episodes, number of previous episodes, and parasitemia (model 3 in Table 3). A mean adjusted decrease of 0.1 g/dL in Hb occurred for each 1 day of illness reported (Table 3). In Tumaco, parasitemia levels were found to be negatively associated with Hb levels only in the unadjusted model. In this site, patients with *P. vivax* malaria had lower Hb levels than those with falciparum malaria, regardless of race. The association between high Hb levels and *P. falciparum* infection was consistently observed independent of age, gender, race, days of illness, previous malaria episodes, number of episodes, and parasitemia (model 3 in Table 4). In Quibdó, an independent association was observed between Hb levels and race. At this site, Afro-descendants with either *P. falciparum* or *P. vivax* malaria infections had lower Hb levels than non–Afro-descendants (11.9 versus 12.9 g/dL). Independent association with the number of previous malaria episodes was also observed. However, these associations were not more significant when adjusted by model 3 (Table 5).

RBC indices are associated with *P. falciparum* infection, days of illness, and previous malaria episodes. Age and gender (male) were positively associated with Htc, MCV, MCH, MCHC, and RBC counts in Tierralta and Tumaco and only Htc and RBC counts in Quibdó (data not shown). After adjusting for potential confounders, such as age, gender, race, parasite species, number of previous malaria episodes, and parasitemia, Htc ($\beta = -0.146$; $P = 0.003$) and RBC ($\beta = -0.151$; $P = 0.003$) counts were negatively correlated with the days of illness in Tierralta only. In contrast, these two indices increased with the number of previous malaria episodes, even after adjusting for the same variables in Tierralta ($\beta = 0.118$; $P = 0.037$ and $\beta = 0.142$; $P = 0.015$, respectively), but this was not observed in either Tumaco or Quibdó (data not shown). After adjusting by model 3, infection with *P. falciparum* was found to be associated with Htc ($\beta = 0.173$; $P = 0.000$) and RBC ($\beta = 0.190$; $P = 0.000$) counts, whereas Htc was negatively associated with parasitemia in Tumaco ($\beta = -0.093$; $P = 0.035$). In Quibdó, a positive correlation was observed between Htc or MCV and the self-reported number of episodes. However, these associations were not found after adjusting by model 3. Other associations with RBC indices were observed, but these were not more significant when adjusted for potential confounders (data not shown).

DISCUSSION

In this study, a moderate prevalence of malaria-related anemia (approximately 36.9%) was observed, with almost no difference between the two *Plasmodium* species, and mild anemia was significantly more frequent with *P. vivax* (1.4 times) than *P. falciparum* infection ($P = 0.018$) in Tumaco only. Because the prevalence of severe anemia was so low

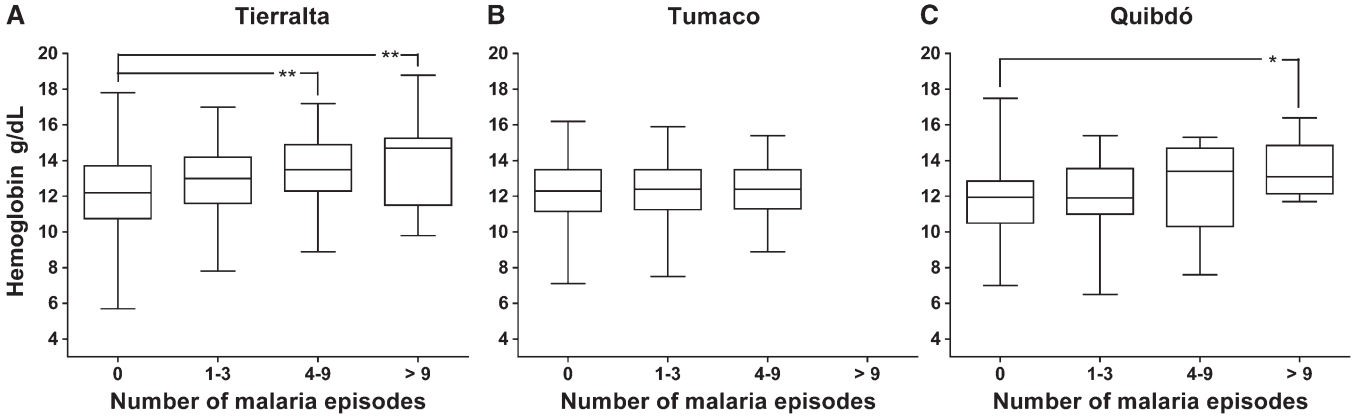

FIGURE 2. Hb levels and numbers of previous malaria episodes. Hb levels according the number of previous lifetime malaria episodes in individuals from (**A**) Tierralta, (**B**) Tumaco, and (**C**) Quibdó. *P value < 0.05; **P value < 0.01.

TABLE 3
Multiple regression models of Hb levels in malaria patients from Tierralta

Variables	Crude		Model 1 ($r_2 = 0.200$)		Model 2 ($r_2 = 0.223$)		Model 3 ($r_2 = 0.250$)	
	β	*P* value	β	*P* value	β	*P* value	β	*P* value
Age (years)	0.304	0.000*	0.288	0.000*	0.268	0.000*	0.229	0.000*
Gender (male)	0.341	0.000*	0.329	0.000*	0.338	0.000*	0.319	0.000*
Race (Afro-descendants)	–0.012	0.819	0.015	0.755	0.022	0.636	0.009	0.853
Parasite species (*P. falciparum*)	0.021	0.700	–0.005	0.917	0.005	0.908	0.013	0.782
Days of illness	–0.172	0.001*			–0.157	0.001*	–0.147	0.002*
Previous malaria episodes (yes)	0.192	0.000*					0.064	0.239
Number of malaria episodes†	0.255	0.000*					0.117	0.034*
Parasites per microliter	–0.027	0.614					–0.015	0.758

Regression coefficients (β) are in grams per deciliter, and *P* values were estimated by linear regression.
*Significant *P* values.
†More than one previous lifetime malaria episode. Model 1 is adjusted by age, gender, race, and species. Model 2 is model 1 adjusted by days of illness. Model 3 is model 2 adjusted by previous malaria episodes, number of episodes, and parasitemia.

(0.3% cases), the influence of the parasite species in this limited number of severe cases could not be determined. None of the volunteers required hospitalization, most likely because of early malaria diagnosis. Data collected by SIVIGILA indicate that most malaria-infected individuals in the country seek diagnosis from healthcare providers within the first 2 days after onset of symptoms.[9] Data presented here indicate that patients attended healthcare facilities for malaria diagnosis within a mean period ranging between 4.0 days in Tierralta and 6.6 days in Quibdó. Nevertheless, because of prompt treatment, there was insufficient opportunity to observe more patients with malarial anemia, regardless of whether mild or severe. Using the adjusted model, it was estimated that there is a 0.1 g/dL Hb decrease per day of illness before diagnosis, which would not be sufficient to result in anemia in the majority of patients. In relation with the longest disease duration reported by patients in Quibdó (Table 1), we hypothesize that this is because of problems associated with access to healthcare services, because the health infrastructure is less developed than in the other study regions.

Although *P. vivax* is the most prevalent malaria parasite in Colombia overall,[9] in this study, the prevalence of *P. vivax* and *P. falciparum* was equal, most likely because of a greater proportion of Duffy (Fy)-negative Afro-descendants in Tumaco and Quibdó and the consequential refractoriness to *P. vivax* infection.[35,36] Indeed, *P. vivax* was most prevalent in Tierralta (92%), whereas *P. falciparum* was predominant in Tumaco (84%) and Quibdó (70%), two areas with a predominantly Afro-descendant population, although with significant Fy polymorphism.[35,36] Higher parasitemias were found in patients infected with *P. vivax* compared with *P. falciparum* (2,939 versus 1,590 parasites/μL, respectively), similar to data previously reported in Colombia.[37] This could be explained by mathematical models studies, in which it has been suggested that, because of the *P. vivax* preference for immature RBCs (reticulocytes), few reticulocytes remain to mature and replenish the older RBCs, causing a high reticulocyte replacement rate that significantly boosts parasitemia in circulation.[38–40] In the three study areas, Hb levels or other RBC indices were found to be associated with demographic and epidemiological variables; these associations were maintained after adjusting for potential confounders. An inverse relationship between days of illness and Hb levels, Htc, and RBC counts was observed in Tierralta. Hb levels were related to the self-reported number of previous malaria episodes in both Tierralta and Quibdó, and Hb levels were modified depending on race and parasite species in Tumaco and Quibdó.

The frequency of malaria-related anemia in this study was within the ranges previously found in Colombia[4,6] and Brazil.[41] Furthermore, this confirms the similar distribution between parasite species reported previously in unstable malaria transmission areas of Brazil, suggesting that infections by either malaria parasite species have similar potential for anemia induction.[41] However, local differences are evident, because in Tierralta, it was observed that non–Afro-descendants infected with *P. falciparum* had lower Hb levels than Afro-descendants, similar to previous reports in Buenaventura.[7] Similarly, in Quibdó, Hb levels in Afro-descendants infected with *P. vivax* were lower than those in non–Afro-descendants, also similar to those observed in Buenaventura.[7] These findings do not have a clear explanation, but we hypothesize that they may be

TABLE 4
Multiple regression models of Hb levels in malaria patients from Tumaco

Variable	Crude		Model 1 ($r_2 = 0.219$)		Model 2 ($r_2 = 0.228$)		Model 3 ($r_2 = 0.235$)	
	β	*P* value	β	*P* value	β	*P* value	β	*P* value
Age (years)	0.130	0.007*	0.185	0.000*	0.185	0.000*	0.184	0.000*
Gender (male)	0.395	0.000*	0.435	0.000*	0.435	0.000*	0.430	0.000*
Race (Afro-descendants)	–0.051	0.286	–0.036	0.401	–0.035	0.408	–0.029	0.501
Parasite species (*P. falciparum*)	0.117	0.015*	0.180	0.000*	0.180	0.000*	0.176	0.000*
Days of illness	–0.013	0.783			–0.015	0.717	–0.018	0.673
Previous malaria episodes (yes)	0.048	0.318					–0.017	0.788
Number of malaria episodes†	0.080	0.095					0.054	0.393
Parasites per microliter	–0.109	0.023*					–0.072	0.096

Regression coefficients (β) are in grams per deciliter, and *P* values were estimated by linear regression.
*Significant *P* values.
†More than one previous lifetime malaria episode. Model 1 is adjusted by age, gender, race, and species. Model 2 is model 1 adjusted by days of illness. Model 3 is model 2 adjusted by previous malaria episodes, number of episodes, and parasitemia.

TABLE 5
Multiple regression models of Hb levels in malaria patients from Quibdó

Variable	Crude		Model 1 ($r_2 = 0.271$)		Model 2 ($r_2 = 0.278$)		Model 3 ($r_2 = 0.283$)	
	β	*P* value	β	*P* value	β	*P* value	β	*P* value
Age (years)	0.322	0.000*	0.302	0.000*	0.310	0.000*	0.295	0.001*
Gender (male)	0.404	0.000*	0.382	0.000*	0.395	0.000*	0.384	0.000*
Race (Afro-descendants)	–0.177	0.037*	–0.124	0.158	–0.119	0.173	–0.121	0.170
Parasite species (*P. falciparum*)	–0.083	0.335	0.037	0.670	0.025	0.772	0.020	0.827
Days of illness	0.002	0.977			–0.090	0.236	–0.085	0.270
Previous malaria episodes (yes)	0.138	0.108					–0.046	0.628
Number of malaria episodes†	0.199	0.020*					0.082	0.391
Parasites per microliter	–0.093	0.279					–0.032	0.678

Regression coefficients (β) are in grams per deciliter, and *P* values were estimated by linear regression.
*Significant *P* values.
†More than one previous lifetime malaria episode. Model 1 is adjusted by age, gender, race, and species. Model 2 is model 1 adjusted by days of illness. Model 3 is model 2 adjusted by previous malaria episodes, number of episodes, and parasitemia.

associated with the specific genetic backgrounds of the ethnic groups studied. Likewise, malaria-related anemia has been associated with nutritional deficiencies, mainly in children,[42,43] as well as intestinal helminthes in endemic regions in Colombia.[24] However, the data are not conclusive, and additional studies are recommended.

The inverse relationship between Hb levels and days of illness observed in Tierralta is consistent with previous observations made in Buenaventura and Tumaco in patients with uncomplicated malaria.[5,7] In relation to days of illness, a misclassification bias is possible because of the way that the parameter was measured; however, this is unlikely because of the acute nature of the episodes, and also, most participants in Tierralta and Tumaco reported previous malaria episodes. Epidemiological data collected by SIVIGILA in Tierralta revealed a total of approximately 12,000 clinical malaria cases between 2010 and 2012, which coincides with the high number of previous self-reported clinical episodes recorded in this study in the same area, thus minimizing potential interpretation bias. The trend toward higher Hb levels with increased numbers of previous malaria episodes observed in Tierralta differed from the association reported in Manaus, Brazil, where Hb levels decreased with previous malaria episodes.[5] Although there is a lack of supporting data, we conjecture that the association found in this study could be caused by the generation of protective immune responses that prevent development of malarial anemia, therefore resulting in higher levels of Hb. Although it has been accepted that clinical immunity requires frequent and repeated experience with malaria, recent studies with volunteers living in areas of low-malaria transmission intensity in Colombia indicated that even a few previous malaria episodes, resulting in low levels of antiparasite antibodies, may be associated with significant protection against clinical malaria as suggested previously.[44,45] Indeed, results presented here indicate that individuals with anemia had a median age of 17 years, whereas the non-anemic individuals had a median age of 22 years, providing indirect evidence of age-dependent immunity to malarial anemia. We believe that the increasing proinflammatory cytokine level that occurs with disease progression could explain the association between Hb levels and days of illness.[46]

Hb and Htc levels in Tumaco were independently associated with parasitemia, but after adjusting for model 3, the association was not found, similar to data reported in Buenaventura[7]; in contrast, Hb levels and RDW were related to parasitemia in Manaus.[5] These results suggest that hemolysis of parasitized RBCs does not play a major role in the mechanisms of anemia observed in the study population. Other factors, including immunological mechanisms with destruction of nRBCs or dysregulation and/or suppression of erythropoiesis, could be the mechanistic factors involved as previously proposed.[2,47] Iron deficiencies have also been identified as an important cause of anemia in populations living in malaria-endemic areas.[4,48] Interestingly, a high percentage of microcytic anemia (60%) has been reported in Colombian adults with malaria.[4] Previous studies have also reported the presence of G6PD deficiency in Colombia, with a prevalence of 12% in patients with *P. falciparum*[49] and 9.5% in patients with *P. vivax*.[50] However, anemia was studied herein before the initiation of the primaquine treatment, and none of the volunteers reported to have had malaria or malaria treatment recently, which indirectly discards G6PD deficiency as a cause of anemia.

In conclusion, these observations suggest that etiologic factors associated with changes in RBC indices in patients with malaria in Colombia seem to be multifactorial. Patients infected with either parasite species seem to have similar potential of developing anemia, although factors specific to each study site seem to modify pathogenesis. Early diagnosis and prompt treatment are likely preventing more frequent and serious anemia in Colombia, with the target age in these low-transmission settings shifting toward adolescents and young adults. Previous malaria experience seems to induce protection against anemia development. Altogether, these data provide information about the distinctive cofactors involved in malarial anemia in local settings with different malaria transmission patterns. Additional studies are warranted to identify the factors that influence development of anemia in specific malaria-endemic regions.

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