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Relation between Vitamin B12 and Folate Status, and Hemoglobin Concentration and Parasitemia during Acute Malaria Infections in Colombia

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Abstract

Anemia is a common complication of human malaria. Since micronutrient deficiencies are highly prevalent in malaria-endemic areas and appear to contribute to anemia etiology, we conducted a cross-sectional study in Tumaco, Colombia, to examine the associations between plasma vitamin B12 or erythrocyte folate concentrations and hemoglobin (Hb) among 96 adults with predominantly *Plasmodium falciparum* malaria. Prevalence of folate and vitamin B12 deficiencies were 26.0% and 26.6%, respectively. There was an inverse, linear relation between folate and Hb concentrations. Adjusted difference in Hb between lowest and highest folate quartiles was 1 g/dL ($p = 0.04$; p, test for trend = 0.01). Vitamin B12 was not associated with Hb concentrations and did not modify the associations between folate and Hb. Incidentally, body mass index (BMI) was inversely associated with parasitemia and risk of clinical malaria. Future longitudinal studies are warranted to determine the potential pathophysiological role of folate in malaria-related anemia.

Keywords

Malaria; Anemia; *Plasmodium falciparum*

1. Introduction

Anemia is one of the most common malaria complications, affecting between 5.2% and 85% of the population in Africa, and 30 to 90% in Latin America (Biemba et al., 2000; Echeverri et al., 2003; Noronha et al., 2000; Zamora et al., 2005). Despite its high prevalence, the

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pathophysiology of malaria-related anemia is poorly understood. Deficiencies in several micronutrients including iron, and vitamins A, B12, and folate are among the leading causes of anemia in low-income countries (van den Broek and Letsky, 2000). These deficiencies are common in malaria-endemic areas, yet their specific contribution to the etiology of malaria-related anemia is unknown and findings from the few studies available are equivocal. In a study carried out in an endemic region of Venezuela, anemia as well as iron and folic acid deficiencies were associated with higher incidence of malaria (Garcia-Casal et al., 2008). However, in another study in Africa, anemia in children with malaria was attributed to under-nutrition rather than to *Plasmodium* infections.

Folate plays a major role in erythropoiesis. Its requirements are increased during malaria infection since hemolysis due to *P. falciparum* stimulates folate-dependent erythroid hyperplasia; thus, malaria could be a risk factor for folate deficiency (Fleming and Werblinska, 1982; Strickland and Kostinas, 1970). However, it has been suggested that folate intake concomitant with malaria treatment decreased the risk of anemia (Tong et al., 1970); another study found no benefit (van Hensbroek et al., 1995). Vitamin B12 is also fundamental for erythropoiesis and some evidence suggests that its absorption may be compromised in *P. falciparum* malaria (Areekul et al., 1972); there is little evidence on its role, if any, on the pathogenesis of malaria-related anemia.

We conducted a cross-sectional study to determine whether erythrocyte folate and serum vitamin B12 levels were associated with hemoglobin concentrations, parasitemia, and clinical malaria in patients from a malaria-endemic region in the Pacific coast of Colombia where both *P. falciparum* and *P. vivax* are prevalent.

2. Patients and Methods

2.1. Study design and population

The study was conducted at Hospital de San Andrés and the outpatient clinic of the Vector Control Program of Tumaco, a community located in the Department of Nariño in the southern region of the Colombian Pacific Coast. Malaria transmission occurs throughout the year, with two small seasonal transmission peaks from April to May and from September to October. The predominant species is *P. falciparum* (90%), followed by *P. vivax* (10%). Communities in this region are racially mixed, with approximately 70% Afro-colombians and 30% Spanish-amerindians.

Between December 2006 and August 2007, adult patients who presented to the outpatient clinic with signs and symptoms of malaria were recruited to the study. Out of a total of 417 patients that were evaluated and found to have positive thick and thin blood smears (TBS), 173 fulfilled the inclusion criteria and agreed to participate in the study. These criteria included age 15-45 years, confirmed malaria diagnosis by TBS, residence within the study area, and ability to read and sign an informed consent. Patients were excluded if they reported any chronic disease. On admission, trained medical personnel inquired about the patients' sociodemographic characteristics, performed a complete clinical examination, and obtained anthropometric measurements. Patients' height to the nearest 0.1 cm was measured with the use of a stature meter (B. Braun Medical, Sheffield, United Kingdom), and weight to the nearest 0.1 kg on a Tanita® HD-351 scale (Tanita Corporation, Tokyo, Japan), using standard techniques. Patients (n=96) were randomly selected for analyses of micronutrient status, and that constituted the final sample size for analyses.

2.2. Laboratory methods

TBS were made using whole blood samples collected by finger-prick and were stained with Giemsa (Moody and Chiodini, 2000). Slides were examined independently for the presence

of malaria parasites by two experienced microscopists. Following administration of standard antimalarial treatment, as recommended by the Colombian Ministry of Social Protection, five mL of blood were drawn in EDTA-Vacutainer® tubes and 5 mL in Vacutainer® tubes without anticoagulant (Becton Dickinson, Franklin Lakes, NJ). Immediately thereafter, a complete blood count including Hb determination was obtained using a Celtα MEK-6318J counter® (NIHON-KOHDEN®, Tokyo, Japan). For folate quantification 1 mL aliquot of whole blood was hemolyzed by dilution in a hypotonic aqueous solution of 1% ascorbic acid; lysates were covered and stored at -20°C until measured. Another aliquot was centrifuged at 400 × g for 5 minutes, and plasma was separated and stored at -20°C until determination of vitamin B12 concentrations. Both quantifications were made at the National Institute of Health (NIH) in Bogotá, using a competitive chemiluminiscent immunoassay in an Advia Centaur analyzer (Bayer Diagnostics, Tarrytown, NY).

2.3. Data Analysis

We first examined the associations of sociodemographic, anthropometric, and clinical characteristics with folate and vitamin B12 concentrations to identify potential confounders of the associations between micronutrients and malaria-related outcomes. Next, differences in the distributions of each micronutrient by categories of the patients' characteristics were analyzed by the Kruskal-Wallis test.

Three malaria-related outcomes were assessed: hemoglobin concentration; parasitemia ($\log_{10}$ transformation); and clinical malaria (high parasitemia, ≥ 4,000/mm^3 with concomitant fever, temperature > 37.5°C) (Bloland et al., 1999; John et al., 2004). The main parameters of interest, folate and vitamin B12 concentrations were grouped by quartiles. Coincidentally, the lowest quartile for each was approximately equivalent to cut-off points that are conventionally used to indicate deficiency (< 305 nmol/L for folate) or marginal status (≤ 221 pmol/L for vitamin B12) (Medicine, 1998). Differences in the distribution of continuous endpoints (hemoglobin and parasitemia) by quartiles of each micronutrient were estimated with the use of linear regression models. For clinical malaria, odds ratios (OR) were estimated with the use of logistic regression. Adjusted estimates were obtained from multivariate models in which known potential confounders were introduced as covariates. We assessed interactions between folate and vitamin B12 concentrations on the outcomes of interest by including cross-product terms in the multivariate models that were tested with the use of the likelihood ratio test. P values < 0.05 were considered to be statistically significant. All analyses were carried out with the Statistical Analysis Software version 9 (SAS® Institute, Cary, NC).

2.4. Ethical considerations

The study protocol was approved by the Institutional Review Board of Universidad del Valle in Cali and the Ethics Committee of Hospital de San Andrés. Written informed consent was obtained from patients before blood was drawn or any additional protected health information was collected. Parents or legal guardians provided consent for patients younger than 18 years of age.

3. Results

Patients were 29 years old on average (range 15-44 years), 60% were women. Thirty-six percent of the women were pregnant (n = 21). Ninety percent of the participants were infected with *P. falciparum*. Mean (±SD) erythrocyte folate and serum vitamin B12 concentrations were 550 ± 344 nmol/L and 319 ± 153 pmol/L, respectively. Prevalence of low folate concentrations (< 305 nmol/L) was 26.0%, while the prevalence of marginal

Vitamin B12 status (≤221 pmol/L) was 26.6%. Only two patients had overt vitamin B12 deficiency (≤148 pmol/L).

Pregnant women had significantly higher mean erythrocyte folate, and significantly lower mean vitamin B12 concentrations compared with either non-pregnant women or with men (Table 1). Folate concentrations were related to BMI following an inverted "J shape" association, whereas vitamin B12 was found to be positively associated with household size.

Mean (± SD) hemoglobin and $\log_{10}$ parasitemia were 10.7 ± 2.3 g/dL and 3.54 ± 0.58, respectively. Forty-one percent of the patients had clinical malaria (confirmed parasitemia concomitant with fever). We noted a statistically significant, monotonic inverse association between folate and mean Hb concentrations, after adjusting for gender, pregnancy status, age, ethnic group, days from the initiation of symptoms, and BMI (Table 2). The adjusted difference in Hb concentrations between the lowest and highest quartiles of folate was 1 g/dL ($p = 0.04$; p, test for trend = 0.01). Further adjustment for parasite species or household size did not change the results. The association was not modified by vitamin B12 status (P for interaction = 0.32). There were no significant associations between erythrocyte folate and parasitemia or clinical malaria. Also, vitamin B12 status was not significantly related to outcome.

BMI was negatively associated with parasitemia and clinical malaria, independent of folate concentrations, gender, pregnant status, age, ethnic groups, and days of illness. Each BMI unit was associated with a mean -0.04 (95% CI = -0.08, -0.001) decrease in $\log_{10}$ parasitemia ($p = 0.046$), and with an 18% lower odds of clinical malaria (OR = 0.82, 95% CI = 0.70, 0.97; $p = 0.02$).

4. Discussion

The goals of this study were to examine the relations between folate or vitamin B12 status and malaria-related outcomes in malaria-infected patients. We found an inverse, linear association between erythrocyte folate concentrations and hemoglobin. BMI was inversely related to parasitemia and risk of clinical malaria. The study was conducted in a region where overall rates of anemia are high [33% in pregnant women according to the National Nutrition Survey of 2005 (Instituto Colombiano de Bienestar Familiar, 2005)], and malaria infection is likely to be an important contributor to hemoglobin status. Persons with hemoglobinopaties, which are common in this region(Moyano and Mendez, 2005), are likely to be underrepresented in our study, since they may be less likely to consult with malaria infection.

We were unable to ascertain whether the inverse association between folate and Hb was restricted to patients with malaria since previous studies have found that high erythrocyte folate was related to severe iron deficiency in the absence of malaria (Saraya et al., 1973). Also, studies of malaria-uninfected schoolchildren from Guatemala (Rogers et al., 2003) and Bogota, Colombia (Arsenault et al., 2009) reported inverse associations between serum folate and hemoglobin concentrations. Similarly, high serum folate concentrations were related to increased prevalence of anemia in elderly persons from the U.S., especially among those with low vitamin B12 concentrations (Morris et al., 2007). Potential mechanisms that could explain this inverse relation are not clear, but may be related to an adverse effect of folate on iron absorption or metabolism.

Few intervention studies have examined the potential impact of folate intake on malaria-related anemia. An early study in adults suggested that the administration of folate or folinic acid together with antimalaria treatment decreased the incidence of anemia (Tong et al., 1970); however, a folic acid supplementation study in children found no evidence of

hematologic benefits (van Hensbroek et al., 1995). Additional intervention studies are clearly warranted to elucidate the role of folate on the hematologic consequences of malaria. These studies should be carefully monitored since higher folate serostatus has been associated with late malaria treatment failure (Dzinjalamala et al., 2005).

We found an inverse association between BMI, an overall indicator of adiposity, and parasitemia or clinical malaria. Protein-energy malnutrition is a known risk factor for adverse outcomes in the course of infections through impairment of several arms of the immune response (Scrimshaw, 2003). Whether leaner adults are at risk of adverse outcomes if they become infected with malaria needs to be confirmed in prospective studies.

Of particular note, the prevalence of folate deficiency in this sample was high (above 25%). This is unexpected considering that wheat flour fortification with folate and other micronutrients has been mandated in Colombia since 1996. A previous study found <1% folate deficiency in school children from Bogota (Arsenault et al., 2009), which suggests there is wide geographical variability in methyl donor nutrient status in this country. Examining access to fortified flour and the quality of fortification are important public health nutrition priorities in this setting.

In conclusion, erythrocyte folate is inversely associated with Hb concentrations in Colombian adult patients infected with *P. falciparum* malaria. Mechanisms to explain this association and its functional consequences remain to be elucidated.

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Table 1

Folate and Vitamin B12 concentrations according to the participants' characteristics

Characteristic	Erythrocyte folate (nmol/L)			Serum vitamin B12 (pmol/L)		
	N	Mean ± SD	p*	N	Mean± SD	p*
Gender / pregnancy status						
Male	38	450 ± 239	0.002	38	332 ± 159	0.05
Female non pregnant	37	501 ± 280		35	343 ± 171	
Female pregnant	21	818 ± 465		21	254 ± 80	
Age (y)	22	578 ± 373	0.64	22	303 ± 154	0.64
<20	33	620 ± 408		32	366 ± 208	
20-29	21	508 ± 291		20	288 ± 75	
30-39	20	448 ± 216		20	290 ± 77	
≥40						
Ethnic group						
Afrocolombian	66	562 ± 368	0.89	65	319 ± 143	0.66
Other	30	524 ± 267		29	318 ± 176	
Residence						
Urban	45	514 ± 350	0.13	43	306 ± 128	0.7
Rural	51	582 ± 339		51	329 ± 172	
Household size						
<5	66	535 ± 359	0.25	65	297 ± 141	0.04
≥5	30	585 ± 312		29	366 ± 170	
Height (cm)						
<160	23	581 ± 387	0.97	23	337 ± 196	0.6
160-169	40	533 ± 309		38	287 ± 98	
≥171	33	549 ± 362		33	342 ± 168	
BMI (kg/m^2)						
<21	18	402 ± 193	0.02	17	320 ± 168	0.32
21-24.9	38	640 ± 349		38	335 ± 143	
≥25	40	532 ± 372		39	302 ± 158	

Characteristic	Erythrocyte folate (nmol/L)			Serum vitamin B12 (pmol/L)		
	N	Mean ± SD	p*	N	Mean± SD	p*
Previous episode of malaria†						
No	65	565 ± 343	0.29	63	322 ± 152	0.54
Yes	28	521 ± 366		28	317 ± 158	
Days of illness, current episode‡						
≤3	62	559 ± 353	0.77	61	305 ± 134	0.31
>3	34	533 ± 331		33	344 ± 182	
Parasite species, current episode						
P. falciparum	86	544 ± 357	0.15	85	321 ± 157	0.81
P. vivax	10	600 ± 211		9	296 ± 103	

*Kruskal-Wallis test

†Whether the patient has had at least one previous clinically diagnosed episode of malaria

‡Number of days from the initiation of symptoms to the first contact day

Table 2

Malaria-related outcomes in relationship to folate and Vitamin B12 concentrations

		Hemoglobin (g/dL)		Log_{10} parasitemia / mm[‡]		Clinical malaria[*]	
	n	Mean ± SD	Difference (95% CI)[†]	Mean ± SD	Difference (95% CI)[†]	%	Odds ratio (95% CI)[†]
Erythrocyte folate (nmol/L)							
<305	25	11.6 ± 2.5	1.0 (0.1, 1.9)	3.51 ± 0.68	-0.11 (-0.44, 0,22)	52	2.48 (0.65, 9.40)
305--439	23	11.2 ± 2.0	1.0 (0.1, 2.0)	3.56 ± 0.46	-0.09 (-0.42, 0.23)	43.5	1.08 (0.30, 3.95)
440--709	24	10.0 ± 2.2	0.2 (-0.7, 1.2)	3.46 ± 0.55	-0.26 (-0.60, 0.07)	29.2	0.46 (0.11, 1.86)
≥710	24	9.9 ± 2.0	Reference	3.63 ± 0.62	Reference	37.5	1
P, test for trend[†]		0.001	0.01	0.62	0.78	0.42	0.1
Serum vitamin B12 (pmol/L)							
<222	25	10.4 ± 2.2	-0.1 (-1.1, 0.8)	3.64 ± 0.60	0.14 (-0.19, 0.47)	32	0.52 (0.14, 1.92)
222--287	21	11.0 ± 2.4	-0.2 (-1.2, 0.8)	3.43 ± 0.58	-0.13 (-0.48, 0.22)	38.1	0.50 (0.13, 1.96)
288--364	24	10.5 ± 2.4	-0.5 (-1.5, 0.4)	3.56 ± 0.63	0.02 (-0.31,0.25)	50	1.15 (0.33, 3.99)
≥710	24	10.9 ± 2.3	Reference	3.51 ± 0.54	Reference	45.8	1
P, test for trend[‡]		0.58	0.96	0.59	0.61	0.23	0.19

[*] Parasitemia ≥4000 with concomitant fever (≥37.5° C)

[†] From multivariate linear (hemoglobin and parasitemia) or logistic regression (clinical malaria) models with indicator variables of each micronutrient plus adjustment covariates that included patients' gender, pregnancy status, age, ethnic group, for categories days from the initiation of symptoms, and body mass index.

[‡] Wald test for an indicator of the ordinal variable that was introduced into the model as a continuous predictor.