Hemoglobin Levels and the Risk of Malaria in Papua New Guinean Infants: A Nested Cohort Study

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Abstract. Studies are available that assess the risk of malaria in accordance to the body's iron store and the systematic iron supplementation of preschool children. However, only a few studies evaluated the temporal association between hemoglobin and malaria and their results are opposing. A total of 1,650 3-month-old Papua New Guinean infants were enrolled in this study and followed-up for 12 months. The risk of malaria was assessed in all children every 3 months and with each episode of fever. The incidence of clinical malaria between 3 and 15 months of age was 249 cases per 1,000 infants per year. After adjustment for potential confounding factors, a decrease of 1 g/dL of hemoglobin was associated with a nonsignificant increase of 11% for risk of malaria infection (hazard ratio, 1.11, 95% confidence interval; CI, 0.99–1.25, $P = 0.076$). Only children with severe anemia (hemoglobin < 8.0 g/dL) at baseline were at higher risk of malaria infection (hazard ratio, 1.72, 95% CI, 1.08–2.76, $P = 0.023$) during the follow-up year compared with the control group (Hemoglobin > 10.0 g/dL). This association was not statistically significant if only clinical malaria episodes were taken into account (hazard ratio, 1.42, 95% CI, 0.77–2.61, $P = 0.26$). Our study suggests that infants with lower hemoglobin levels are not protected against malaria infection. Further research that examines the risk of malaria in relation to both hemoglobin and iron store levels would be important to better understand this complex interaction.

INTRODUCTION

In 2013, about 190 million cases of malaria occurred worldwide provoking 584,000 deaths.¹ Children are particularly susceptible to malaria, which is one of the leading causes of death in children of less than 5 years.² Africa and Southeast Asia are the areas where anemia has the highest prevalence with affection present in two-thirds of preschool-age children; therefore, representing a severe public health problem. In Papua New Guinea, the proportion of preschool-age (< 5 years old) children with anemia (hemoglobin < 110 g/L) is estimated at about 25.7–86.5%.³ Anemia is caused by a wide range of etiologies. Iron deficiency is the leading cause of anemia and malaria is one of the major causes, especially in west sub-Saharan Africa, where it accounts for 25% of all prevalent anemia.⁴ Despite iron deficiency being a major cause of anemia, hemoglobin alone is not an optimal marker of this as it is not sensitive or specific enough, and other markers are preferred.^{5,6}

In 2006, a large study performed in Tanzania found a positive relationship between malaria mortality and routine supplementation with iron and folic acid in preschool children.⁷ Subsequently, two meta-analyses^{8,9} were performed and concluded that iron supplementation does not adversely affect children living in malaria-endemic areas and maintained the use of routine iron supplementation. One of the studies indicated that in areas without access to malaria treatment and prevention services, an increased risk of malaria could not be excluded. These meta-analyses draw attention to the inability to assess how some factors such as baseline hemoglobin might affect the risk of malaria and as a result did not lead to a general conclusion regarding the use of iron and folic acid combined supplementation. Thus, the World Health Organization (WHO) recommends universal

iron supplementation for preschool- and school-aged children in areas where anemia prevalence is above 20%, in association with measures to prevent malaria.¹⁰ However, the risk of routine iron supplementation for children with repleted iron stores in malaria endemic areas remains a matter of debate.^{11,12} Studies that assess the relationship between iron stores and malaria risk could bring some answers to these uncertainties. A few observational studies have been performed in children living in malaria-endemic areas to assess the relation between malaria risk and iron stores using ferritin alone or in combination with C-reactive protein (CRP) or transferrin. Three of them showed a protective effect of iron deficiency,^{13–15} and one study showed no relation between iron stores and risk of malaria.¹⁶ The assessment of this relationship is difficult because inflammatory status increases iron markers such as ferritin and can cause anemia.^{17–19} Residual confounding effects cannot be excluded.¹⁵

The association between malaria episodes and hemoglobin levels have not been reported in trials on iron supplementation.⁹ Only a few studies assessing the association between hemoglobin, regardless of its origin, and malaria risk have been published, and their results were contradictory.^{20,21}

The present nested cohort study offers the opportunity to strengthen the currently limited knowledge on the relationship between hemoglobin and malaria risk. In agreement with the results obtained in most of the aforementioned studies using ferritin alone or ferritin in combination with CRP or transferrin as a marker of iron deficiency,^{13–15} we hypothesized that low hemoglobin values would be associated with a lower incidence of malaria in Papua New Guinean infants. However, as hemoglobin alone is not an adequate marker of iron deficiency, our results cannot be compared with the results obtained from studies using other markers such as evaluating body iron stores.

METHODS

Study objectives. This study aimed to investigate the relationship between hemoglobin levels and the subsequent risk

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of malaria in Papua New Guinea in 3-month-old infants and specifically to assess if hemoglobin levels were associated with 1) the incidence of clinical malaria, 2) the frequency of repeated episodes of clinical malaria, and 3) the density of *Plasmodium* parasites counted in the first infection.

Trial design, sites, and study participants. We used data from the Intermittent Preventive Treatment (IPTi) randomized trial in Papua New Guinea to perform this nested cohort study.²² In this placebo-controlled randomized trial, 1,650 Papua New Guinean infants aged 3 months (± 1 month) were enrolled from June 2006 to May 2010. The trial took place on the north coast of Papua New Guinea. Twenty villages situated within a 400 km² coastal area, 30–60 km north of Madang town, took part in the study. A second site in the Sepik province was also used and included around 20 villages. The average annual precipitations are over 3,000 mm and there is a short dry season from June to October. This region is considered to have hyperendemic malaria.^{23,24} Unlike sub-Saharan Africa, where *Plasmodium falciparum* is the most predominant parasite and similarly to areas of the Southwest Pacific, Southeast Asia, and the Americas, malaria infection by *Plasmodium vivax* occurs more frequently.^{25–29} The study villages were serviced by two health centers and several aid posts that were responsible for delivering the Expanded Program on Immunization in monthly outreach clinics. As the national malaria program distributed free long lasting insecticide treatment in late 2008, untreated nets were predominantly used. More details on the enrollment and study location are available in Senn et al.²²

The study was carried out in accordance with Good Clinical Practice (ICH GCP E6) guidelines and externally monitored by two independent monitors and a Data Safety Monitoring Board. The Papua New Guinea Medical Research Advisory Committee (MRAC number 05.20) approved the study. The trial was registered on <http://www.clinicaltrials.gov> (number NCT00285662) and formed part of the IPTi Consortium (<http://www.ipti-malaria.org>).³⁰

Inclusion and clinical study procedures. The first contact of the parents or guardians of potential study participants with the study staff took place when the child was 1–2 months of age at the routine Expanded Program on Immunization clinics. The inclusion of a child meeting inclusion criteria into the study was performed at the 3-month-old clinical visit after obtaining at least one written parental consent. Children were included in the nested study if they fulfilled the following criteria: 1) permanent resident of the area, 2) aged 3 (± 1) months old at the time of inclusion, 3) no disability, 4) no chronic illness, 5) no known allergy to study drugs, 6) hemoglobin > 5.0 g/dL, 7) no severe malnutrition according to the Papua New Guinea national guidelines.³¹ Children with a positive blood smear for malaria at enrollment or a reported history of illness within two weeks before baseline enrollment were excluded from our analysis.

At inclusion, a concise medical history, including the presence of acute illness and possible disabilities was carried out and a physical examination was done. Subsequent routine visits were performed at 6, 9, and 12 months. The preventive malaria treatment or placebo was given at inclusion (3 months) and then during the routine visits at 6, 9, and 12 months. During visits, a clinical routine examination and vaccination were performed and blood was collected to check for parasitemia. The capillary blood hemoglobin was measured with a

HemoCue 201[®] machine at 3 and 15 months and after each episode of illness during the follow-up.

Throughout the study period the detection of disease episodes was performed by a passive case detection system maintained at the three health centers and the three aid posts where study participants received free-of-charge treatments. The study staff assessed each episode of illness using a standard case report form. For all cases of history of fever in the past 48 hour or an axillary temperature 37.5°C, a rapid malaria diagnostic test (ICT Combo) was performed and blood was afterward collected to assess parasitemia (the result was not used for clinical management), and hemoglobin levels were measured. The infants positive for malaria on the rapid diagnostic test were treated with artemether/lumefantrine (Coartem). It was initially decided that children with a hemoglobin value of < 8 g/dL should be generally treated with oral iron (ferrous sulfate, 5 mg/kg daily for 6 weeks) and folate supplementation. Health center staff treated study participants who attended after hours based on presumptive diagnosis. In these cases, the study staff recorded the reason for admission and performed a finger prick, when possible, to check parasitemia and to measure hemoglobin values the next day.

Defining anemia (exposure). There is no international consensus for the definition of anemia in 3-month-old children. According to WHO, for children aged 6 to 59 months, a level of hemoglobin of 10.9 g/dL diagnoses anemia.³² Orkin et al.³³ reported a mean value of 11.2 g/dL hemoglobin at 2 months old, two standard deviations below the mean of 9.4 g/dL, and a mean value of 12.2 g/dL at 4 months with two standard deviations below the mean of 10.3 g/dL. Therefore, we decided to use a threshold of 10.0 g/dL for anemia. Anemia was graded as mild, moderate, or severe, with thresholds of 9.0, 8.0, and under 8.0 g/dL of hemoglobin, respectively. In this study, the mean hemoglobin value is used for the sensitivity analysis. It refers to the mean of all hemoglobin values available for a study participant up to one month before the

FIGURE 1. Study inclusion and exclusion criteria flowchart.

TABLE 1
Characteristics of infants at the time of entry

	Hemoglobin at 3 months				Chi2 test <i>P</i> value
	Control	Anemic			
	≥ 10 g/L	9.9–9 g/L	8.9–8 g/L	< 8 g/L	
	(<i>N</i> = 430)	(<i>N</i> = 492)	(<i>N</i> = 330)	(<i>N</i> = 51)	
	33.0% (<i>n</i>)	37.8% (<i>n</i>)	25.3% (<i>n</i>)	3.9% (<i>n</i>)	
Sex (male)	51.6 (222)	51.8 (255)	54.9 (181)	43.1 (22)	<i>P</i> = 0.442
Age at entry					<i>P</i> = 0.241
61–75 days	12.1 (52)	7.7 (38)	7.3 (24)	9.8 (5)	–
76–90 days	33.3 (143)	36.6 (180)	33.6 (111)	29.4 (15)	–
91–105 days	36.7 (158)	36.0 (177)	40.6 (134)	33.3 (17)	–
106–120 days	17.9 (77)	19.7 (97)	18.5 (61)	27.4 (14)	–
Weight at entry					<i>P</i> < 0.001
< 5 kg	14.4 (62)	10.8 (53)	16.4 (54)	33.3 (17)	–
5–5.9 kg	38.1 (164)	44.9 (221)	46.2 (152)	33.3 (17)	–
6–6.9 kg	35.8 (154)	37.2 (183)	32.5 (107)	23.5 (12)	–
≥ 7 kg	11.6 (50)	7.1 (35)	4.9 (16)	9.8 (5)	–
Received iron/folate supplement					<i>P</i> < 0.001
Yes	2.6 (11)	3.7 (18)	3.6 (12)	15.7 (8)	
Health center					<i>P</i> = 0.215
Mugil	70.2 (302)	64.7 (318)	68.5 (226)	60.8 (31)	–
Maprik	29.8 (128)	35.3 (174)	31.5 (104)	39.2 (20)	–
Treatment arm of intervention (%)					<i>P</i> = 0.433
Placebo	32.1 (138)	35.8 (176)	34.8 (115)	39.2 (20)	–
Sulfadoxine-pyrimethamine plus amodiaquine	34.6 (149)	29.1 (143)	33.9 (112)	35.3 (18)	–
Sulfadoxine-pyrimethamine plus artesunate	33.3 (143)	35.2 (173)	31.2 (103)	25.5 (13)	–

first episode of malaria infection. The hemoglobin was measured at 3 and 15 months and after each episode of illness during the follow-up. The markers of body iron stores were not available in this study.

Case definition. Blood slides were examined by light microscopy with 200 thick-film fields. Slides were scored as light-microscopy-positive for an individual *Plasmodium* species, if the species was independently detected by at least two microscopists. Parasite densities were recorded as the number of parasites per 200 white blood cells and converted to the number of parasites/microliter assuming 8,000 white blood cells/microliter.³⁴ More details on laboratory procedures are available in Senn et al.²²

In the present study, two concepts were defined, malaria infection and clinical malaria. Malaria infection includes all the episodes where malaria parasites were detected (in the blood slide or in absence of a blood slide, positive rapid diagnostic test) during an episode of fever (reported or measured, more than 37.5°C), or during a routine visit. Clinical malaria includes only the episodes where malaria parasites were detected during an episode of fever.

Statistical methods. The survival analysis compared the risk of malaria infection over 12 months across groups of

infants with different levels of anemia, (mild, moderate, or severe), starting at three months of age to those with normal hemoglobin values (≥ 10 g/dL) at the same age. The hazard proportionality assumption was verified using Therneau & Grambsch test of the nonzero. Hazard ratios were then computed using Cox regression. Breslow method was used for ties. The linearity of association for different severities of anemia was assessed using the likelihood ratio test comparing Cox regression model with anemia category, entered as a nominal value and the same model with hemoglobin, entered as a continuous value. Significance was set at *P* < 0.05. The sensitivity analysis was conducted using the participant's mean hemoglobin value instead of the hemoglobin value obtained at the 3-month-old visit. The analyses were performed in Stata version 13.³⁵ To rule out coding errors, the entire analysis was coded twice independently.

RESULTS

Data were made available from 1,605 children. A total of 1,303 were included in this analysis. Study exclusion criteria are listed in Figure 1.

TABLE 2
Cox regression model for risk of malaria infection between baseline hemoglobin groups

	Unadjusted model			Adjusted model*		
	Hazard ratio	95% Confidence interval	P value	Hazard ratio	95% Confidence interval	P value
Reference: ≥ 10 g/dL						
9.0–9.9 g/dL (mild)	1.00	0.79–1.26	0.989	0.98	0.78–1.25	0.895
8.0–8.9 g/dL (moderate)	1.10	0.85–1.42	0.461	1.13	0.87–1.46	0.353
< 8.0 g/dL (severe)	1.58	1.00–2.51	0.051	1.73	1.08–2.77	0.023

Bold values indicate *P* < 0.05.

* Adjusted for: age, sex, weight, supplementation with iron and folic acid, treatment arm of intervention.

FIGURE 2. (A) Cumulative hazard of malaria infection. (B) Cumulative hazard ratio of clinical malaria.

The participants' baseline characteristics were similar across hemoglobin groups in terms of sex, age, treatment group attribution, and health service center. Children with severe anemia were more likely to receive iron and folate supplementation. A direct relationship between weight and hemoglobin level was noticed. The details on characteristics of infants at entry are provided in Table 1.

Hemoglobin values were normally distributed with a mean value of 9.52 g/dL (95% CI, 9.47–9.58). At the time of entry, the prevalence of anemia defined by a hemoglobin value of < 10 g/dL was 67.0% ($N = 873$). In total, 49 infants received iron and folic acid supplements.

Cases of malaria. Malaria infection. Without any adjustment, lower hemoglobin levels were related to a non-significant increased risk of malaria infection (Table 2, Figure 2a). The model was adjusted for age, weight, sex, having received iron and folate substitution during the follow-up, treatment group attribution, and health service center. After adjustment, the risk of malaria remained higher with lower hemoglobin levels and became significant (hazard ratio, 1.72, 95% confidence interval; CI, 1.08–2.76, $P = 0.023$) comparing the most severe anemic group (hemoglobin < 8g/dL) with the control group (nonanemic, hemoglobin ≥ 10 g/dL) group (Table 2). The adjusted model revealed a significantly decreased risk of malaria infection in children who received iron and folate substitution at entry or during the follow-up for hemoglobin of < 8 g/dL at entry or during the follow-up (hazard ratio, 0.50, 95% CI, 0.27–0.95, $P = 0.035$).

When hemoglobin values were analyzed individually and not in subgroups, each decrease of 1 g/dL of hemoglobin was associated with a nonsignificant increase of risk of malaria infection in the unadjusted (hazard ratio, 1.09, 95% CI,

0.97–1.22, $P = 0.136$) and adjusted model (hazard ratio, 1.11, 95% CI, 0.99–1.25, $P = 0.076$) (Table 3).

Clinical malaria. The overall incidence of clinical malaria between the ages 3 months to 15 months was 249 new cases per 1,000 infants per year. Clinical malaria refers to a positive malaria test obtained during an episode of fever. When clinical malaria was used as the outcome, the hazard of clinical malaria increased nonsignificantly with anemia severity (Figure 2B, Table 4). Contrary to the previous analysis using malaria infection as an outcome, the hazard ratio for all anemia groups remained nonsignificant. For the group with severe anemia, the hazard ratio for clinical malaria was 1.42 (95% CI, 0.77–2.61, $P = 0.26$).

In addition to the time-to-event analyses, an assessment of the absolute number of clinical malaria episodes during the follow-up year was performed. Children were divided into four different groups in accordance to their number of clinical malaria episodes during the follow-up year; no significant differences in hemoglobin values were found (Supplemental Figure 1). During episodes of clinical malaria, no significant relationship between baseline hemoglobin and log blood parasite density was detected (Supplemental Figure 2).

Sensitivity analysis. A sensitivity analysis was performed using the mean hemoglobin value instead of the baseline value to define children's group assignment. The risk of malaria infection was increased in every anemic group. This risk increased nonsignificantly in each group, except in the moderate anemia group (Table 5), where the increase was significant with (1.81, 95% CI, 1.37–2.40, $P < 0.001$) or without adjustment (1.63, 95% CI, 1.24–2.14, $P < 0.001$). When clinical malaria was used as the outcome instead of malaria infection, the risk of malaria was significant only after adjustment for the moderate anemia group (hazard ratio, 1.45, 95% CI,

TABLE 3
Cox regression model for risk of malaria infection according to differences in absolute levels of hemoglobin

	Unadjusted model			Adjusted model*		
	Hazard ratio	95% Confidence interval	P value	Hazard ratio	95% Confidence interval	P value
Hemoglobin (g/dL)	1.09	0.97–1.22	0.136	1.11	0.99–1.25	0.076

Hazard ratios correspond to the increased risk of malaria for every drop of g/dL for hemoglobin.

* Adjusted for: age, sex, weight, supplementation with iron and folic acid, treatment arm of intervention.

TABLE 4
Cox regression model for the risk of clinical malaria between baseline hemoglobin groups (without adjustment)

	Unadjusted model			Adjusted model*		
	Hazard ratio	95% Confidence interval	P value	Hazard ratio	95% Confidence interval	P value
Reference: ≥ 10 g/dL						
9.0–9.9 g/dL (mild)	0.95	0.72–1.26	0.739	0.93	0.70–1.24	0.638
8.0–8.9 g/dL (moderate)	1.03	0.76–1.40	0.835	1.07	0.78–1.46	0.665
< 8.0 g/dL (severe)	1.26	0.69–2.3	0.449	1.42	0.77–2.61	0.265

* Adjusted for: age, sex, weight, supplementation with iron and folic acid, treatment arm of intervention.

1.03–2.05, $P = 0.035$) (Table 6). Finally, there was no significant relationship between mean hemoglobin and log blood parasite density performed during the episodes of clinical malaria.

DISCUSSION

Our results suggest that if an association between hemoglobin and malaria risk exists, this would be in favor of an increased risk of malaria infection with lower hemoglobin values. Our study thereby rules out any clinically significant protective effect of anemia on malaria infection. Nevertheless, despite lower levels of hemoglobin associated with a higher risk of malaria, this association was not always statistically significant. A decrease of 1 g/dL of hemoglobin was associated with an increased risk of malaria infection of 11% (hazard ratio = 1.11, $P = 0.076$). The risk of malaria infection was increased by 72% in the severe anemia group compared with the control group (hazard ratio, 1.72, $P = 0.023$). Our results show that risk of malaria infection was 50% lower in children that received iron and folate at entry or during the follow-up (hazard ratio = 0.50, $P = 0.035$). The criteria for receiving iron and folate supplementation were hemoglobin of < 8.0 g/dL at entry or during an episode of illness. However, the substitution was not systematically performed in all cases of hemoglobin < 8.0 g/dL. For example, in the severe anemia group, only 15.7% of the infants were substituted. A direct relationship between weight and levels of hemoglobin was observed and may reflect different maternal and perinatal factors, such as maternal anemia or prematurity that may be associated with both conditions. Unfortunately, these parameters were not collected.^{36,37} The secondary objectives assessed the association between hemoglobin levels with the incidence of clinical malaria, with the frequency of repeated episodes of clinical malaria and with the density of *Plasmodium* parasites counted during the first infection. None of these analyses showed a significant association.

The strengths of this study are its large size: data from 1,303 infants were included in the analysis. Furthermore, children were regularly assessed during the one-year follow-up through routine visits and to document every case of illness,

a passive case detection was ensured by an efficacious net of outlying clinics and health centers where medical management was delivered for free. However, despite the large sample size that minimizes random error, selection bias may have influenced our results. We cannot exclude that parents of infants who were anemic more easily visited the medical centers than parents with healthy infants at study inclusion. This may have falsely reduced the time until first diagnosis of malaria in children with anemia at study inclusion. The main analysis was performed using a single hemoglobin value measured at three months of age; however, this value may not adequately reflect the actual level of baseline hemoglobin from 3 to 15 months of age. To mitigate this limitation, a sensitivity analysis was performed using the mean value of hemoglobin. This study was not able to inform on the etiology of anemia. Finally, our study did not explore the impact of maternal, perinatal, or other hidden parameters that could have an impact on both hemoglobin levels and the subsequent risk of malaria. In light of these limitations, an absence of biological gradient and the lack of association are plausible. Only two studies reported an association between hemoglobin and subsequent risk of malaria. As in our case, one of them showed that anemic children were marginally more susceptible to malaria than nonanemic children after adjustment for confounding factors. This retrospective study was performed on 1,072 children between 6 and 36 months in Gambia.²⁰ The other study on the same topic found opposite results to ours: they observed that children with low birth hemoglobin had lower malaria rates at follow-up.²¹

Finally, most of the studies assessing the relationship between iron stores and risk of malaria used ferritin alone or ferritin combined with CRP and/or transferrin as markers of the body's iron stores and concluded that low iron stores protect against malaria.^{13–16} Hemoglobin levels were not reported in these studies. These results cannot be compared with our results as causes of anemia are multiple and hemoglobin alone is not an optimal marker of iron deficiency as it is not sensitive or specific enough.^{5,6}

Our results suggest that if an association between hemoglobin and malaria risk exists, this would be in favor of an

TABLE 5
Cox regression model for the risk of malaria infection between mean hemoglobin groups (without adjustment)

	Unadjusted model			Adjusted model*		
	Hazard ratio	95% Confidence interval	P value	Hazard ratio	95% Confidence interval	P value
Reference: ≥ 10 g/dL						
9.0–9.9 g/dL (mild)	1.17	0.92–1.49	0.194	1.17	0.92–1.49	0.196
8.0–8.9 g/dL (moderate)	1.63	1.23–2.13	< 0.001	1.81	1.37–2.40	< 0.001
< 8.0 g/dL (severe)	1.18	0.62–2.26	0.608	1.44	0.74–2.78	0.281

Bold values indicate $P < 0.05$.

* Adjusted for: age, sex, weight, supplementation with iron and folic acid, treatment arm of intervention.

TABLE 6
Cox regression model for the risk of clinical malaria between mean hemoglobin groups (without adjustment)

	Unadjusted model			Adjusted model*		
	Hazard ratio	95% Confidence interval	P-val	Hazard ratio	95% Confidence interval	P-value
Reference: ≥ 10 g/dL						
9.0–9.9 g/dL (mild)	1.09	0.83–1.46	0.521	1.10	0.83–1.46	0.519
8.0–8.9 g/dL (moderate)	1.28	0.91–1.79	0.157	1.45	1.03–2.05	0.035
< 8.0 g/dL (severe)	1.33	0.64–2.75	0.442	1.66	0.79–3.49	0.182

Bold values indicate $P < 0.05$.

* Adjusted for: age, sex, weight, supplementation with iron and folic acid, treatment arm of intervention.

increased risk of malaria infection with lower hemoglobin values. There is, therefore, a very small probability that low hemoglobin protects against malaria infections. This study may also indicate that hemoglobin levels and iron stores have different correlations with malaria. Further research that assesses the risk of malaria in relationship with hemoglobin and iron store levels combined would be important for a better understanding of this complex interaction.

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