

The Effect of *Plasmodium falciparum* Parasitemia on Serum Iron Status in Patients Treated with Quinine

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Abstract

This study aimed to assess the iron status, by hemoglobin (Hb), hematocrit (Hct), serum iron (SI) and serum ferritin (SF), in forty-five falciparum malaria patients without hematological disturbances. Patients with 5 parasitemia levels were compared and treated with quinine 600 mg intravenously every 8 hours for 2 days, followed by 600 mg orally every 8 hours for 5 days. No sex bias was observed. The mean parasitemia was 206.7 parasites/ μ l and 57.8% revealed parasitemia level 2. The mean Hb and SI values before and after treatment showed significant differences at $p = 0.017$, and 0.009 , respectively. The Hb, Hct, SI, and SF values were not significantly related to the onset of disease and Body Mass Index (BMI). The averages of erythropoietin (EPO) in five patients before and after treatment were 23.4 mU/ml and 7.3 mU/ml, respectively.

Keywords: *Plasmodium falciparum*, parasitemia, iron status, quinine

Introduction

Malaria continues to be a major health problem in endemic countries and a global concern. It is endemic in 91 countries and >40% of the world's human inhabitants live in areas where there is a risk of malarial infection. In 1996, the World Health Organization (WHO) estimated 500 million clinical cases of malaria and up to 2.7 million malaria-attributable deaths occur each year [1]. Anemia is the most important cause of mortality and morbidity in patients with acute

complicated *Plasmodium falciparum* malaria infections, particularly in young children and in non-immune adults. In highly malarious areas, therefore, it is almost inevitable that malarial infection will be associated with anemia. The presence of parasites is detectable in less than 30% of all cases showing hemolysis. The effects of *P. falciparum* infection involve many organ systems, and it is extremely difficult to determine which of a multitude of potential factors are interacting to produce the severity of anemia that often accompanies this condition. In general, anemia may cause a reduction in circulatory rate, which will affect the neurological system, heart rate regulation, heart abnormality, enlargement and failure. Anemia occurs if there is defective production of red cells, or if there is an increasing rate of loss, either by hemolysis or bleeding. The cause of anemia has not been clearly explained. At schizogony, the infected erythrocytes rupture

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and release numerous merozoites to undergo the next asexual cycle. The red cells are destroyed and anemia develops rapidly in severe cases [2-3]. The administration of drugs, such as quinine, may lead to immune-mediated hemolysis. Severe malaria infection causes liver and kidney damage and disturbances leading to decreased synthesis of erythropoietin, which regulates erythropoiesis [3]. The WHO has defined severe anemia in malaria as a hemoglobin level <50 g/L (5 g/dl) or hematocrit $<15\%$, in the presence of a *P. falciparum* parasitemia $>100,000$ parasites/ μ l, with a normochromic normocytic blood film [4]. Schapira *et al* [5] reported asymptomatic parasitemia remaining after poor response to full antimalarial treatment may lead to life-threatening anemia [6]. Malaria and anemia, due to iron deficiency, are two leading causes of morbidity worldwide. Little is known about the relative contribution of *P. falciparum* infection and iron status to the etiology of malaria anemia in endemic areas. Malaria may also contribute to iron status, with resultant anemia, by reducing iron absorption during acute episodes and through sequestration of iron in malaria pigment. This study aimed to assess and compare parasitemia levels due to the iron statuses of patients who were treated with quinine, and those who were not.

Materials and methods

Study design

A cross-sectional study was carried out during the period October 2001 to May 2002. The study population was falciparum malaria-infected patients aged 15-60 years admitted to Sangklaburi Hospital, Kanchanaburi and Suanphung Hospital, Ratchaburi, Thailand. The Ethics Board of Mahidol University approved the study. All patients were informed of the objectives of the study, and of the protection of human rights. All subjects of the study signed for consent for human subject research before enrollment into the study.

Subjects screening

The patients' medical history, physical examination and peripheral blood smears (thick and thin films) were obtained. Patients positive for falciparum malaria were interviewed using the

questionnaire. Stool examination for hookworm infection was also done. The study population was selected according to the inclusion criteria.

Inclusion criteria

Subjects included into the study were falciparum malaria patients aged 15-60 years, both male and female, in a good conscious state, admitted to the hospital and treated with quinine. They were screened for hematological disorders of kidney, liver, spleen and bone marrow.

Exclusion criteria

Excluded from the study were malaria patients who had a history of malaria infection or those who had taken an antimalarial drug, in the past year, cerebral or severe malaria patients, had a history of being a blood donor or blood transfusion in the previous four months, or a history of illness, including diabetes mellitus, peptic ulcer, hyperthyroidism, hepatitis/cirrhosis, aplastic anemia, glucose-6-phosphatase-dehydrogenase deficiency. Thalassemia and rheumatoid arthritis cases were also excluded.

Laboratory investigations

Parasite density was estimated by counting the number of parasites relative to the number of erythrocytes in a thin film and expressing the result as count/ μ l scans about 10,000 red blood cells. Since the average oil immersion field at 1,000 magnification contains about 200 red cells, this meant looking at 50 such fields [7]. Blood samples of 12 ml were collected before drug administration (2 ml for determination of hemoglobin and hematocrit, and 10 ml for serum iron and serum ferritin). The specimens for serum iron and serum ferritin determination were centrifuged at 3,000 rpm for 20 minutes within 1 hour after the sampling process was done. The plasma supernatant was carefully transferred to test tubes and stored at -20°C until the laboratory investigation was performed. Hematocrit was determined by microhematocrit method, and serum iron by colorimetric method using a spectrophotometer with chemical reagent (protein precipitant solution, and chromogen solution). This method is based on the International Committee

Standard of Hematology (ICSH) recommended assay [8]. The reference range is 50-150 µg/dL, with an average of 125 µg/dL for men, and 100 µg/dL for women. A mixed acid reagent was used to release the transferrin-bound iron, reduce the ferric iron to ferrous, and to precipitate the serum proteins. The ferrous iron in the remaining supernatant was color-developed using buffered bathophenanthroline sulphonate and the absorbance of the resulting pink solution was read at 535 nm. A standard iron solution and a 'blank' (iron-free water) were treated similarly. An immunoturbidimetric assay, which was based upon the reaction between ferritin in the sample, and latex-covalently-bound anti-ferritin antibodies assessed serum ferritin. Ferritin values were determined turbidimetrically, using fixed-time measurement with sample blank correction. The relationship between absorbance and concentration permits a multipoint calibration with a measuring range between zero to 500 ng/ml. The measuring temperature was 37 °C. A set of standards was used to calibrate the assay (Multipoint calibration mode). An automated analyzer was programmed according to the parameters. Using a commercial kit (Roche, Germany) serum ferritin was qualified. Briefly, fifty microliters of sample were transferred to a tube containing anti-ferritin antibody latex reagent. The mixture was agglutinated when it formed an antigen antibody complex. The sample was measured turbidimetrically with an absorbance of 700 nm. The concentration of serum ferritin was calculated with standardization for the non-linear calibration curve on the Hitachi 704. The normal concentration range is 15-300 µg/L, but it is dependent on age and sex. The range for men is referenced at 30-300 µg/L and at 15-200 µg/L for women. The serum erythropoietin level was measured by ELISA technique. The erythropoietin enzyme immunoassay was a commercially available enzyme immunoassay (Boehringer Mannheim Biochemica, Mannheim, Germany) based on the double-antibody sandwich method. Microliter wells, precoated with polyclonal antibodies to erythropoietin, were incubated with standard or test serum, washed, and reincubated with anti-erythropoietin monoclonal antibodies conjugated

with peroxides. Sodium perorate and 2,2-acino-di-3-ethyl-benzthiazoline sulfonate were added, resulting in the formation of a color complex, the absorbance of which was determined using a microplate reader. The parasite density was counted by thin film and then calculated into parasites/ml. The study population was administered with 600 mg intravenous quinine every 8 hours for two days, followed by 600 mg oral quinine every 8 hours for five days. All samples were repeated after the clinical conditions were improved.

Statistical analysis

Data for general characteristics, results of parasitemia, iron status and erythropoietin, were analyzed by descriptive statistics (mean, standard deviation, and percentage). The mean comparisons between iron status before and after treatment were analyzed using the t-test. The relationships between parasitemia levels and different means of iron status were analyzed by using one-way Analysis of Variance (ANOVA).

Results

General characteristics

During the period October 2001 to May 2002, 521 cases were naturally infected with *P. falciparum*, *P. vivax*, *P. ovale* and mixed infections of *P. falciparum* and *P. vivax* at Sangklaburi Hospital and Suanphung Hospital (OPD and IPD). There were 248 *P. falciparum* cases, 99 *P. vivax* cases, 1 *P. ovale* case, 7 cases of mixed infections of *P. falciparum* and *P. vivax* at Sangklaburi Hospital. At Suanphung Hospital, 144 cases were *P. falciparum* and 22 cases were *P. vivax*. Within the 2 hospitals, the highest number of patients (159 cases) was reported in May, while the lowest (34 cases) was in October. Six cases were excluded, one of thalassemia and five of hookworm infestation. Of the total number of cases, only 45 met the inclusion criteria and were selected for this study, comprised of 37 cases (82.2%) at Sangklaburi Hospital and 8 cases (17.8%) at Suanphung Hospital. There were 26 males and 19 females, and the ratio of male to female was 1.36:1. The mean age was 35.6 years, and ages ranged from 15 to 56 years. The majority were Mon (33.3%), followed by Thai (28.9%), Karen (28.9%) and Burmese (8.9%). The majority of the patients were married

(77.8%), and remainder were single (15.5%) and widowed (6.7%). The major occupation was general employee (48.9%), followed by agriculture (31.1%), unemployed/house keeper (15.6%), and merchant (4.4%).

Parasitological response

Parasitemia was counted by thin film. The parasite density was 42-1,210 parasites/ μ L. The mean parasite density was 206.7 parasites/ μ L. The majority (57.8%) of the study population was in level 2 (101-200 parasites/ μ L) [9] (Table 1). The parasite clearance time was two to six days; mean parasite clearance time was 3.0 days. 42.2% of patients were negative at day 3, while 31.1%, 20%, 4.4% and 2.2% had negative parasitemia at days 2, 4, 5 and 6, respectively (Fig 1).

Hematological response before and after

quinine treatment

Anemia was defined by a hemoglobin level 12 g/dL in females and 13 g/dL in males. Twenty-nine cases (64.4%) had anemia before treatment and 33 cases (73.3%) had anemia after treatment. Serum iron before and after quinine treatment ranged between 31-204 μ g/dL and 48-198 μ g/dL. Mean serum iron before and after quinine treatment was 85.5 ± 39.9 and 101.1 ± 34.9 , respectively. Before treatment, 22 cases (48.9%) had low serum iron, and 8 (17.8%) had high serum iron. After treatment, 16 cases (35.6%) had low serum iron, and 13 (28.9%) had high serum iron (Fig 2). Serum ferritin before and after quinine treatment ranged between 69 to 2,850 μ g/L and 90-1,730 μ g/L, while mean serum ferritin before and after treatment were 563.6 ± 603.7 and 529 ± 426.9 , respectively. Before treatment, 24 cases (53.3%) had high serum ferritin, and after treatment, 30 cases (66.7%) had

Table 1 Number and percentage of the study population by parasitemia level before quinine treatment (n = 45).

Parasitemia level (parasites/ μ L)	No. of cases	%
Level 1 (less than 100)	10	22.2
Level 2 (101- 200)	26	57.8
Level 3 (201 400)	5	11.1
Level 4 (401 –800)	1	2.2
Level 5 (801- 1,600)	3	6.7

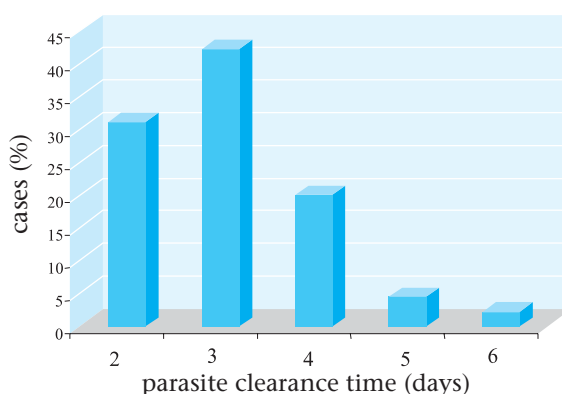


Fig 1 Parasite clearance time (days) of the percentage of falciparum malaria patients (n = 45).

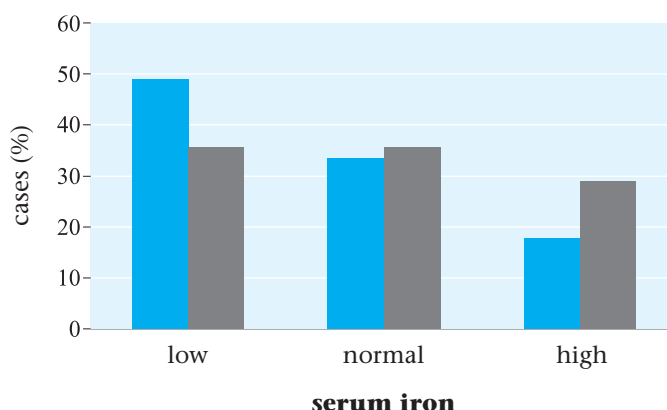


Fig 2 Percentage of cases by serum iron concentrations in falciparum malaria patients (low, normal and high concentrations) before (■) and after (■) quinine treatment (n = 45).

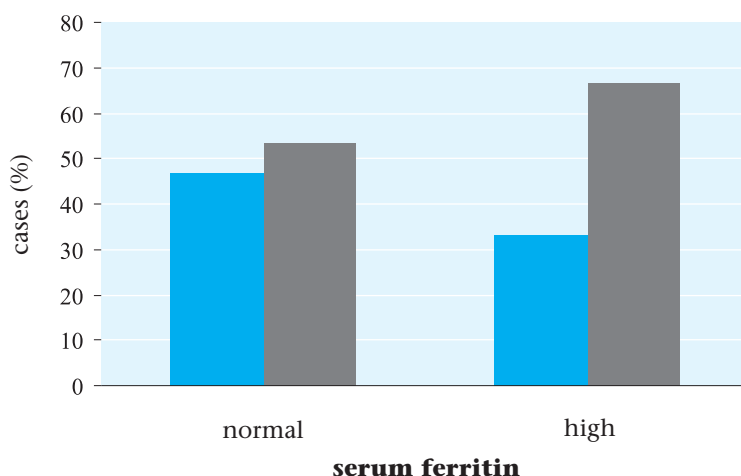


Fig 3 Percentages of cases by normal and high concentrations of serum ferritin before (■) and after (■) treatment (n = 45).

high serum ferritin (Fig 3).

Iron status and parasitemia before and after quinine treatment

Means and standard deviations for hemoglobin, hematocrit, serum iron, and serum ferritin before and after quinine treatment, are presented. The values of hemoglobin before and after quinine treatment were 8-16.7 g/dL and 7-16 g/dL, while mean hemoglobin before and after treatment was 11.9 ± 2.2 and 11.5 ± 1.9 ,

respectively. The hematocrit values before and after quinine treatment were 24-50 vol% and 21-48 vol%, respectively. Means for hematocrit before and after treatment were 35.7 ± 6.5 and 34.5 ± 5.9 , respectively. The values for the range of serum ferritin level were wide so they were transferred into a logarithm. It revealed that hemoglobin and serum iron before and after quinine treatment were significantly different ($p = 0.017$, 0.009 at significant level 0.05), while hematocrit and serum ferritin levels before and after quinine treatment

were not significantly different as shown in Table 2. There were no significant differences in parasitemia levels and means of hemoglobin, hematocrit, serum iron and serum ferritin, with $p = 0.700, 0.681, 0.675$, and 0.773 , respectively.

The mean differences by Pearson correlation among parasitemia, hemoglobin, hematocrit, serum iron and serum ferritin in falciparum patients, before and after quinine treatment are shown in Table 3. There were significantly differences between the different means of

hemoglobin and hematocrit in patients before and after quinine treatment with $p < 0.01$.

The duration of onset of malaria disease ($X = 3.3$ days) due to the existence of symptoms was recorded. The relationship between hemoglobin, hematocrit, serum iron and serum ferritin levels before and after quinine treatment, and the duration of onset of disease was analyzed by using the regression statistical method. The duration of onset of disease before quinine treatment had 30.6% relationship to the duration of onset of

Table 2 Mean, standard deviation, minimum, maximum and comparison of mean Hb (g/dL), Hct (vol%), serum iron, SI ($\mu\text{g/dL}$) and serum ferritin, SF ($\mu\text{g/L}$) in falciparum patients, before and after quinine treatment ($n = 45$).

Variables	Mean	SD	Min	Max	t	p
Hb [†]	11.9	2.2	8	16.7		
Hb [‡]	11.5	1.9	7	16.0	1.647	0.017*
Hct [†]	35.7	6.5	24	50		
Hct [‡]	34.5	5.9	21	48	1.708	0.095
SI [†]	85.5	39.9	31	204		
SI [‡]	101.1	34.9	48	198	- 2.722	0.009*
SF [†]	0.4	2.6	69	2850		
SF [‡]	0.3	2.6	90	1730	- 1.516	0.137

* Significant difference at $p < 0.05$; [†] before treatment; [‡] after treatment.

Table 3 The correlation of mean differences among parasitemia, Hb (g/dL), Hct (vol%), serum iron, SI ($\mu\text{g/dL}$) and serum ferritin, SF ($\mu\text{g/L}$) in falciparum patients, before and after quinine treatment ($n = 45$).

	Parasitemia	Hb [§]	Hct [§]	SF [§]	SI [§]
Parasitemia	1.000	-0.039	-0.038	-0.016	0.068
	0	0.799	0.804	0.915	0.659
Hb [§]	-0.390	1.000	0.998*	0.051	0.101
Hct [§]	0.799	0	0	0.741	0.509
	-0.380	0.998*	1.000	0.105	0.109
SI [§]	0.804	0	0	0.493	0.475
	-0.016	0.051	0.105	1.000	0.168
SF [§]	0.915	0.741	0.493	0	0.270
	0.068	0.101	0.193	0.168	1.000
	0.569	0.509	0.475	0.227	0

* Significant difference at $p < 0.01$.

[§] Mean differences before and after quinine treatment.

disease after quinine treatment ($r^2 = 0.094$). The duration of disease had a 9.4% effect on the values for hemoglobin, hematocrit, serum iron, serum ferritin, before and after quinine treatment. It meant that when the duration of onset of disease changed, it effected iron status, with SE = 1.83. There were no significant relationships to the values for hemoglobin, hematocrit, serum iron and serum ferritin before and after quinine treatment, at P-value > 0.05 (Table 4).

Body mass index (BMI) had a 27.7%

relationship to the duration of onset of disease after quinine treatment ($r^2 = 0.077$). The body mass index had a 7.7% effect on the hemoglobin, hematocrit, serum iron, serum ferritin values before and after quinine treatment. This meant that body mass index had no effect to the values for iron status, with SE = 2.71. There were no significant relationships to the values for hemoglobin, hematocrit, serum iron and serum ferritin before and after quinine treatment, at P-value > 0.05 (Table 5).

Table 4 Relationship between onset of disease and Hb (g/dL), Hct (vol%), serum iron, SI ($\mu\text{g/dL}$) and serum ferritin, SF ($\mu\text{g/L}$) in falciparum patients, before and after quinine treatment (n = 45).

Unstandardized coefficients	B	Standardized coefficients		t	p
		SE	Beta		
Hb [†]	3.633	10.378	4.526	0.350	0.728
Hb [‡]	-1.932	3.190	-2.190	-0.060	0.548
Hct [†]	-1.223	3.454	-4.570	-0.354	0.725
Hct [‡]	0.699	1.066	2.371	0.655	0.517
SI [†]	0.001	0.001	0.382	1.277	0.210
SI [‡]	-0.000	0.001	-0.202	-0.615	0.542
SF [†]	-0.001	0.009	-0.043	-0.202	0.841
SF [‡]	0.007	0.010	0.151	0.765	0.449

$R^2 = 0.094$, $F = 0.466$, $p = 0.872$ by one-way ANOVA test.

Table 5 Relationship between Body Mass Index (BMI) and Hb (g/dL), Hct (vol%), serum iron, SI ($\mu\text{g/dL}$) and serum ferritin, SF ($\mu\text{g/L}$) in falciparum malaria patients (n = 35).

Unstandardized coefficients	B	Standardized coefficients		t	p
		SE	Beta		
Hb [†]	1.714	17.295	1.380	0.099	0.922
Hb [‡]	1.941	5.222	1.410	0.372	0.713
Hct [†]	-0.537	5.747	-1.298	-0.094	0.926
Hct [‡]	-0.689	1.732	-1.508	-0.398	0.694
SF [†]	-0.000	0.001	-0.047	-0.126	0.901
SF [‡]	0.000	0.002	0.167	0.401	0.691
SI [†]	-0.021	0.017	-0.334	-1.229	0.230
SI [‡]	0.011	0.015	0.117	0.748	0.461

$R^2 = 0.077$, $F = 0.271$, $p = 0.970$ by one-way ANOVA test.

Five samples had very high levels of serum ferritin. They were tested for serum erythropoietin (EPO). The normal range is 1.6-34 mU/ml. Two cases had serum erythropoietin levels higher than the normal range. Four cases had decreased serum erythropoietin level and one case had an increase. The mean serum erythropoietin levels before and after quinine treatment were 23.4 ± 17.2 (6.3-46.9) mU/ml and 7.3 ± 5.4 (1-15.4) mU/ml, respectively.

Discussion

The proportion of malaria cases found to be higher in males (57.8%) than females (42.2%). The ratio between males and females was 1.4:1. Likewise, many previous studies reported that malaria could be found more in males than females. In Thailand, many studies indicated that malaria incidence rates for males were higher than those for females. Men had a tendency to suffer from malaria attacks and they were more likely than women to work in the jungle where they would frequently be exposed to mosquito bites. We found the age group of 15-29 years had the largest number of infections (37.8%); these subjects were employed and exposed to malaria infection while migrating through malarious areas. Almost all malaria cases were Mon, Karen and Thai.

Many reports have implicated the lack of parasitemia clearance, resulting from ineffective chloroquine therapy, as a factor increasing the incidence of malarial anemia. Hemoglobinopathies and nutritional factors may have contributed to lingering anemia in some patients. Inadequate production of erythropoietin or a prolonged elevation in the levels of certain inflammatory cytokines may be the most likely explanations for a retardation in the reconstitution of normal hemoglobin concentrations. Malaria anemia, associated with both high and low-density parasitemia, has been described. Higher density is associated with symptoms (fever) and acute anemia; asymptomatic lower-density parasitemia may also result in anemia, particularly if the parasitemia persists for prolonged periods due to ineffective treatment. Pasvol *et al* [10] found no correlation between age and time for parasite clearance, and that patients treated with a high-

dose quinine regimen cleared parasites significantly faster than those given low-dose quinine regimen, thus indicating *in vivo* that the drug concentration affected the rate of parasite clearance.

In this study, anemia was defined by a hemoglobin level of less than 12 g/dL in females and less than 13 g/dL in males. Before and after quinine treatment, 64.4% and 73.3% of the patients, respectively, were anemic. Mean hemoglobin levels before and after treatment had no significantly different, the level before treatment was higher than after treatment ($X = 11.9, 11.5$). These results showed that the patients were still had hemolysis, which may result from direct destruction of parasitized erythrocytes. The value of hematocrit was continued to decrease. The patients who were not anemic, were possibly had high baseline levels of hematocrit, so the decreased hematocrit level did not appear lower than the normal range. In this study, we could not determine baseline hematocrit level before infection. This study was similar to previous studies; patients who were not significantly anemic at admission presented a rapid fall in hemoglobin level, eventually, but the parasites did not appear. The fall of hemoglobin level continued for a period, while the lowest level showed at the end of the first week or at ten days, after which reticulocytosis occurred, during which the level would return to normal, within three or four weeks. There was no significant relationship between the values of hemoglobin, hematocrit before and after treatment with quinine and the duration of onset of disease.

This study found no differences between parasitemia level and hemoglobin level possibly due to low parasitemia, in contrast to Beales [11] and Newton *et al* [12], who reported a relationship between the degree of parasitemia and decreasing hemoglobin level. It had been believed that the greater the number of red cells destroyed, the greater the number of parasitized cells were lost from the circulation. Mean serum iron before quinine treatment was lower than after quinine treatment ($X = 85.5, 101.1$), but in the normal range. These findings accord with Abdalla [13], who found normal mean serum iron in Gambian

children with malaria. They reported secondary anemia (low serum iron) in some patients with acute malaria. Our study contrasts with the study in Ghanaian children, as the mean serum iron level was below normal in the asymptomatic malaria children, but normal in the uninfected group. Snow *et al* [14] found similar serum iron in children who subsequently developed symptomatic or asymptomatic malaria. The patients had hemolysis of the red blood cells.

Mean serum ferritin before quinine treatment was higher than after quinine treatment, but it was not significantly different, which is similar to many studies that found higher serum ferritin levels in malaria patients. The serum ferritin level increased in infection and hemolysis. The parasitemia level in this study was not higher when the patients had high parasitemia, which contrasted with the finding of Snow *et al* [14] that mean serum ferritin was high in children who had high levels of parasitemia. Erythropoietin is a glycoprotein produced primarily by the kidney and is considered the principal factor regulating red blood cell production. Hypoxia is the major stimulus of EPO production. Serum EPO levels must be expressed in relation to hematocrit, and should be evaluated in relation to the degree of anemia. Inadequate EPO production in patients suffering from acute *P. falciparum* malaria might be an indicator of prolonged anemia. This study assessed serum EPO before and after quinine treatment in five patients. The serum erythropoietin values in most patients was in the normal range. The mean EPO before quinine treatment was higher than after quinine treatment. Four cases had decreased serum erythropoietin levels and one case had an increased level. However, serum EPO could not be measured in all 45 cases. This study partly concluded that malaria patients produced inadequate EPO which agree with the study of Burchard *et al* [15] and Burgmann *et al* [16], who found lower EPO and inadequate EPO production in-patients with malaria. The anemia associated with *P. falciparum* alone is quite complex and poorly understood. Malarial anemia may result from direct destruction of parasitized erythrocytes. However, the degree of anemia that often develops

in parasitemic individuals cannot be accounted for solely by hemolysis of parasitized erythrocytes. Estimation of hemoglobin, hematocrit, serum iron and serum ferritin in blood, level of malarial parasitemia and clinical condition of the patient should become additional parameters in the management of malaria patients at primary-care level, particularly in deciding upon appropriate drug supplementation.

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