

Serum transferrin receptor concentration indicates increased erythropoiesis in Kenyan children with asymptomatic malaria¹⁻⁵

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ABSTRACT

Background: Serum transferrin receptor concentrations indicate both erythropoietic activity and the deficit of functional iron in the erythron. In contrast with serum ferritin concentrations, serum transferrin receptor concentrations are not or are only marginally influenced by the inflammatory response to infection.

Objective: We assessed iron status and examined the relation between serum transferrin receptor concentrations and malaria in children aged 2–36 mo who were asymptomatic for malaria.

Design: This was a community-based cluster survey ($n = 318$).

Results: Prevalences of malaria, anemia (hemoglobin concentration <110 g/L), iron deficiency (serum ferritin concentration <12 $\mu\text{g/L}$), and iron deficiency anemia were 18%, 69%, 53%, and 46%, respectively. Malaria was associated with lower mean hemoglobin concentrations (92.7 compared with 104.1 g/L; $P = 0.0001$) and higher geometric mean serum concentrations of transferrin receptor (11.4 compared with 7.8 mg/L; $P = 0.005$), ferritin (21.6 compared with 11.9 $\mu\text{g/L}$; $P = 0.05$), and C-reactive protein (12.5 compared with 6.8 mg/L; $P = 0.004$). There was no evidence for an association between serum concentrations of C-reactive protein and transferrin receptor. Children with malaria had higher serum transferrin receptor concentrations than expected for the degree of anemia, even after adjustment for inflammation indicated by serum C-reactive protein concentration quartiles ($P = 0.02$).

Conclusions: Our findings are consistent with the notion that malaria-induced hemolysis is accompanied by increased erythropoiesis. Serum transferrin receptor concentration is not useful for detecting iron deficiency in individuals with malaria. Individuals with high concentrations of serum C-reactive protein or similar acute phase reactants should be excluded from analysis if serum ferritin concentrations <12 $\mu\text{g/L}$ are to be used to measure iron deficiency in malaria-endemic areas. *Am J Clin Nutr* 2001;74:767–75.

KEY WORDS Iron deficiency, anemia, ferritin, transferrin receptors, C-reactive protein, falciparum malaria, helminthiasis, preschool children, Kenya, community survey

INTRODUCTION

Iron deficiency and malaria are likely causes of anemia in African children (1–4), but little is known about the pathogenic mechanisms involved. Malaria leads to hemolysis, impaired ery-

thropoiesis (5), and possibly iron sequestration and iron deficiency (6). The relative contributions of these mechanisms likely varies with the burden, activity, and duration of infection. Ferritin is an iron storage protein, and serum ferritin concentrations <12 $\mu\text{g/L}$ are highly predictive of iron deficiency, defined by the absence of iron stores (7). Serum ferritin also reacts as an acute phase protein (8). Chronic infections generally cause anemia and a shift of iron distribution from functional toward storage compartments (9), as reflected by increased serum ferritin concentrations. The resulting hypoferrremia may occur despite the presence of sufficient iron stores (9). Until recently, it was believed that anemia of infection is primarily due to impaired release by macrophages of iron from degraded hemoglobin (10, 11). More recent evidence suggests rather that the prime cause of anemia of infection is decreased responsiveness of erythroid cells to erythropoietin and relatively impaired erythropoietin production, both under the influence of inflammatory cytokines (12, 13).

Observations that serum soluble transferrin receptor (sTfR) concentrations are not or are only marginally influenced by the inflammatory response to infection (14, 15) are consistent with the notion that hypoferrremia in infection is a nonspecific consequence of activation of inflammatory cytokines (12) and that erythroblasts are not deficient in iron (16). As reviewed by Feelders et al (15), sTfR concentrations are closely correlated

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²HV, CEW, and FJK were responsible for the study design and interpretation of results; HV was responsible for data collection and analysis; PN assisted in detailed planning of the field work and data collection; JB advised on statistical analyses; and YB conducted the biochemical analyses and assisted in the interpretation of those results.

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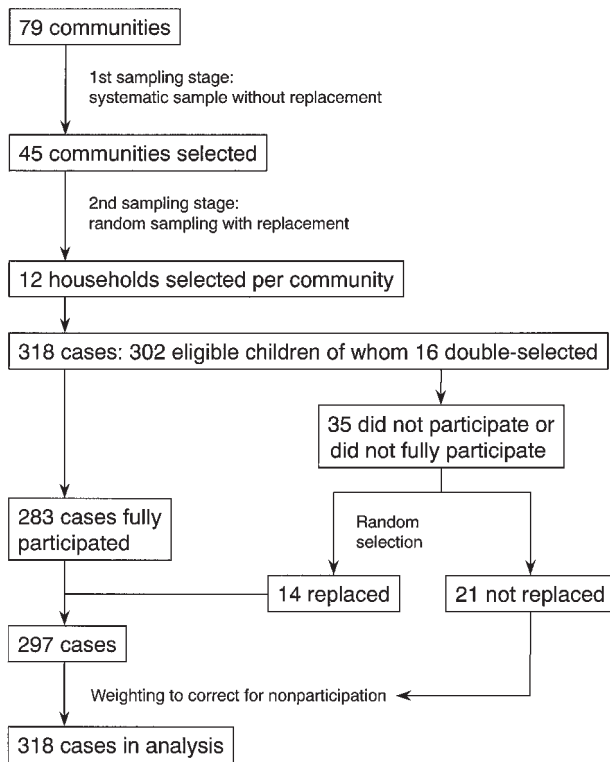


FIGURE 1. Framework for selection and analysis.

with the number of receptors expressed on the surface of erythroblasts, where they transport transferrin-bound iron into the cell. An expansion of the erythroid mass results in increased sTfR concentrations, and increased transferrin receptor expression occurs on the surface of iron-deficient erythroblasts. Hence, sTfR concentrations measure both erythropoietic activity and the deficit in functional iron in the erythron.

We hypothesized that malaria-induced hemolysis results in increased erythropoietic activity under the influence of stimulated erythropoietin production, and that these and possibly other mechanisms lead to increased sTfR concentrations. We also hypothesized that serum ferritin concentrations are increased in malaria independent of their association with hemoglobin concentration. Using data from a survey of preschool children in an area with seasonal malaria transmission, we assessed the children's iron status and tested whether our data are consistent with these hypotheses.

SUBJECTS AND METHODS

Study area

The present study was conducted during the first annual rainy season (April–June) of 1997 in 3 administrative areas (Kathe-kani, Muthingiini, and Mangelete) of Mtito Andei Division, Kenya. These areas together comprise ≈ 720 km² at an altitude of 800–900 m above sea level, located halfway on the road and rail link between Nairobi and Mombasa. The inhabitants belong almost exclusively to the Akamba tribe and live in widely scattered homesteads. Like elsewhere in rural Africa, communities are defined as administrative units, rather than as physically rec-

ognizable groups of houses. The vast majority of the population are engaged in subsistence farming, with maize and beans cultivated as staple foods, and consume a diet poor in meat. Malarial infection reported at the clinical facilities in the area is exclusively due to *Plasmodium falciparum*. There is no active malaria control program in the area, and no epidemiologic or entomologic studies of malaria have been conducted previously.

Study population and sampling procedures

The target population comprised children living in the study area and aged 2–36 mo, with no sickness with associated symptoms suggesting malaria or anemia, as reported by mothers or caregivers. Children were selected by using a cluster sampling procedure (17), incorporating modifications proposed by Bennett et al (18) and Brogan et al (19). A household survey, conducted with the assistance of local government administrators, community leaders, and auxiliary health workers (including community health workers, traditional birth attendants, and traditional herbalists) listed a total of >40 000 inhabitants spread over 79 communities in the study area. For enumeration purposes and as the basic sampling unit, we defined a household as a group of persons living on the same premises and whose food is prepared by the same person or persons. Each household was identified by the name of its head, and the number of its members was listed. The sample was drawn in 2 successive stages (Figure 1). At the first stage, a systematic sample of 45 communities was drawn from a north-to-south ordered list of all 79 communities (clusters) in the study area, with sampling probability proportional to size and excluding urban centers (Mtito Andei town).

From each of the selected communities, 12 households were randomly sampled with replacement, and for each of these households, the resident children were listed together with their dates of birth as ascertained from the child health card. All resident children thus identified with no symptoms of malaria or anemia and within the desired age range were selected for the study ($n = 302$). Children who migrated with only some household members between the time of the census and the time of examination were considered not to be eligible and were excluded without replacement. Children who migrated with all household members, were still missing after repeated visits, or had parents who refused consent were replaced when possible by children from randomly selected households within the same community.

Field procedures

Mothers and their children were invited to prearranged meetings held in or near their resident communities. Containers for collection of stools were distributed by the auxiliary health workers the day before the clinical examination and stools were examined for parasites on the day of the medical examination. For those children who could not produce stools on that day, mothers were asked to collect and deliver samples the next day. Community leaders, locally active auxiliary health workers, and parents of eligible children were informed in their preferred language about the purpose and procedures of the study and prior written consent was obtained from the parents. Children were treated as deemed necessary on completion of the survey. Because such treatment occurred after all observations were made and samples collected, this had no influence on the data collected. The study was approved by the African Medical and Research Foundation and the Kenya Medical Research Foundation, whose ethical standards were followed.

Capillary blood samples were taken by using finger or heel punctures and a portion was collected in containers (Microtainer without additives; Becton Dickinson, Franklin Lakes, NJ). Serum was stored in liquid nitrogen (-196°C) within 12 h after blood collection and kept on solid carbon dioxide or frozen (-79°C) during and after transport to Europe for subsequent biochemical analysis.

Anemia was defined as a hemoglobin concentration <110 g/L (20). The accuracy of the hemoglobinometer (HemoCue Inc, Ångelholm, Sweden) was checked every 4 h during the measurements by using a control cuvette. Thick and thin blood films were stained by using Field's stain and were examined by experienced technicians on the day of collection and cross-checked independently later. At least 100 high power ($\times 1000$) fields of the thick films were examined for malaria parasites before a slide was considered negative. Stool samples were collected and examined on the day of stool production in saline solution. Additional stool samples were used to make Kato-Katz smears (21) and were examined for *Schistosoma* infections or stored in 10% formal saline until examined for the presence of hookworm, *Ascaris lumbricoides*, or *Trichuris trichiura* (21). Intestinal worm infections or schistosomiasis were defined as a positive stool test result for a particular parasite in any stage of its development.

Ferritin concentrations were measured by radioimmunoassay (Ciba-Corning, Brussels). Concentrations of sTfR were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis). C-reactive protein concentrations were measured as indicators of the activity of infection by a standard turbidimetric method. The intra- and interassay CVs were as follows: serum ferritin concentration, 3.7–5.9% and 4.9–9.1%, respectively; sTfR concentration, 4.3–7.1% and 5.4–6.4%, respectively; and serum C-reactive protein concentration, 3.1–4.4% and 2.6–5.7%, respectively.

Response and missing values

A total of 302 children were eligible and selected for study, of whom 16 were double-selected at the second sampling stage (Figure 1). Observations for these 16 children were weighted twice. Thus, 318 children were included in the study, of whom 35 did not participate or fully participate for the following reasons: refused consent ($n = 26$), not home or temporarily absent ($n = 7$), and hospitalized for burns ($n = 2$). Of these 35 children, 14 were replaced by random selection, which brought the total number included in the analysis to 297. In the case of nonparticipating children who were not replaced, weighting was used to maintain the validity of assuming an equal probability sample. Thus, observations on those who participated within the same cluster were inflated by weighting with a multiplication factor calculated as the number of selected children in that cluster divided by the number of participating children (18, 19). This brought the number back to 318 children; sample sizes reported below this value are due to missing values. Only a limited volume of capillary blood could be collected; in the biochemical analysis, priority was given to measuring concentrations of sTfR, ferritin, and C-reactive protein (in this order). Thus, missing values occurred more frequently with the last 2 variables.

Statistical analysis

Data were entered into a computer, cleaned up, and managed by using SPSS for WINDOWS (version 7.5; SPSS Inc, Chicago) and were analyzed by using SUDAAN for WINDOWS (version 7.5.2a;

Research Triangle Institute, Research Triangle Park, NC), assuming 2-staged cluster sampling with replacement at the first sampling stage. The variance estimates under this assumption do not consider that clusters were sampled from a finite population. Thus, the SEs and CIs reported here are overestimates, and the statistical tests are conservative in detecting existing associations. Recalculation of the variance estimates for the descriptive statistics with use of ordered lists of clusters (22) led to only marginally smaller values (data not shown).

To assess the associations between malaria and indicators of iron status and to determine the activity of the infection, we compared children with and without malaria with respect to their hemoglobin concentration and serum concentrations of sTfR, ferritin, and C-reactive protein, respectively. Distributions of serum concentrations of sTfR, ferritin, and C-reactive protein were normalized by decimal logarithmic transformation before analysis.

The associations between age and indicators of iron status were assessed by estimating the prevalences of anemia, iron deficiency, and iron deficiency anemia (hemoglobin concentration <110 g/L and serum ferritin concentration <12 $\mu\text{g/L}$) in 3 age groups (2–11, 12–23, and ≥ 24 mo). Prevalences for anemia were given separately for children with and without malaria. Because serum ferritin concentrations are higher when infection is present, this was controlled for in 2 different ways: by excluding children with malaria and by excluding those with serum C-reactive protein concentrations ≥ 8 mg/L. Individuals without infections usually have serum C-reactive protein concentrations <8 mg/L (23).

To assess whether the activity of infection was associated with indicators of iron status, we compared children with relatively low and high serum concentrations of C-reactive protein with respect to their hemoglobin concentration and serum concentrations of sTfR and ferritin. Values of sTfR concentration were normalized by decimal logarithmic transformation for use as a dependent variable in linear regression models and were re-expressed in their natural units for reporting. Malaria-associated changes in the rate of erythropoiesis may depend on immune status and therefore on age (24). This was examined by testing the product term malaria \times age class in a multiple regression model that also included malaria and age class as main terms.

To further explore the association between sTfR concentration and malaria, geometric mean sTfR concentrations were estimated for each of the cross-tabulated categories of the variables malaria and hemoglobin concentration class. Multivariate linear regression models were subsequently used to assess this association with adjustment for possible effects of inflammation. Thus, a dependent variable indicating log-transformed sTfR concentrations was modeled as a function of hemoglobin concentration class, malaria, and serum C-reactive protein concentration quartiles. Exclusion of the latter variable did not result in different conclusions or substantially different effect estimates. Hemoglobin concentration was entered as a categorical variable with 3 classes (<100 , 100–110, and ≥ 110 g/L) so as not to impose linear relation with the outcome. Cutoffs for hemoglobin concentration were chosen to optimize sample size distribution between categories. Sex, age (categorized as above), and intestinal worm infections were considered as potential confounding variables, but their inclusion led to similar conclusions and coefficient estimates for the effects of malaria and hemoglobin concentration class (not shown), so that these terms were deleted in the final model (model 1; $n = 88$).

Similar procedures were followed to assess the association between serum ferritin concentrations and malaria. This model

TABLE 1
Characteristics of the study population used in the multiple regression analysis¹

Variable	Population estimate
Age (mo) ²	18.9 [318]
Percentage male (%)	55 [173]
Percentage female (%)	45 [144]
Hemoglobin (g/L) ³	102.1 (75.0, 122.7) [318]
Serum soluble transferrin receptor (mg/L) ⁴	8.3 (4.7, 14.5) [224]
Serum ferritin (μg/L) ⁴	13.1 (3.1, 54.5) [199]
Serum C-reactive protein (mg/L) ⁴	7.5 (3.4, 16.6) [91]
Prevalence of anemia: hemoglobin concentration <110 g/L (%)	69.1 [318]
Prevalence of severe anemia: hemoglobin concentration <80 g/L (%)	6.9 [318]
Prevalence of iron deficiency: serum ferritin concentration <12 μg/L (%)	41.5 [199]
Prevalence of inflammation: serum C-reactive protein ≥8 mg/L (%)	37.8 [91]
Prevalence of fever: axillary temperature ≥37.5°C (%)	6 [317]
Prevalence of malaria (%)	17.6 [318]
Prevalence of worm infections (%)	
<i>Ascaris lumbricoides</i>	4.4 [283]
<i>Trichuris trichiura</i>	2.5 [243]
<i>Schistosoma mansoni</i>	0.4 [241]
Hookworm	0 [318]

¹*n* in brackets.

²Median (children were uniformly distributed over the selected age range of 2–36 mo).

³Arithmetic \bar{x} (5th, 95th percentiles).

⁴Geometric \bar{x} (5th, 95th percentiles).

(model 2; *n* = 90) adjusted for possible effects on serum ferritin concentration of inflammation by including serum C-reactive protein concentration quartiles. Although exclusion of age class did not substantially affect the effect estimate of malaria on serum ferritin concentration, this variable was retained in the model because iron stores are known to rapidly decline during and after infancy as a result of growth and associated hemodilution (25).

RESULTS

Descriptive statistics

Descriptive statistics for the study population are provided in **Table 1**. All reported malaria infections were the result of *P. falciparum*. The prevalence of malaria (17.6%) indicated a population of children exposed to seasonal, unstable malaria. Anemia was highly prevalent but moderate in degree. Iron defi-

ciency, as measured in the total sample, occurred in 41.5% of children. The highest prevalence for any intestinal infection was for *A. lumbricoides* (4.4%). The parasite load for this roundworm was low, ranging between 20 and 1100 eggs/g stool. No hookworm infection was found.

Univariate analyses

Compared with those without malaria, children with malaria had substantially lower hemoglobin concentrations, higher sTfR concentrations, higher ferritin concentrations, and higher C-reactive protein concentrations (**Table 2**). The prevalences of children with serum ferritin concentrations <12 μg/L were 45.1% and 22.4% in children without and with malaria, respectively (difference: 22.6%; 95% CI: 6.6%, 38.7%; *P* = 0.008). Children with malaria also had slightly higher axillary temperatures than did those without malaria (36.9 compared with 36.5°C; difference 0.4°C; 95% CI: 0.1, 0.6°C; *P* = 0.01).

Hemoglobin concentrations were not associated with sex (not shown). Of the 3 age classes examined, children aged 12–23 mo had the highest prevalence of anemia, iron deficiency, and iron deficiency anemia (**Table 3**). The prevalence of anemia increased with malaria in all age categories. Exclusion of children with serum C-reactive protein concentrations ≥8 mg/L led to higher estimated prevalences of iron deficiency and iron deficiency anemia than did exclusion of those with malaria.

Compared with those with low serum C-reactive protein concentrations (<8 mg/L), children with high serum C-reactive protein concentrations (≥8 mg/L) had greatly increased serum ferritin concentrations (geometric mean of 25.1 compared with 9.4 μg/L; difference: 15.7 μg/L; 95% CI: 4.4, 36.1 μg/L; *P* = 0.002). Concentrations of sTfR were somewhat higher in children with high serum concentrations of C-reactive protein (9.3 compared with 7.6 mg/L), but this difference was not significant (difference: 1.6 mg/L; 95% CI: -4.0, 4.2 mg/L; *P* = 0.13) and disappeared altogether after adjustment for malaria (not shown). Serum concentrations of C-reactive protein did not correlate with hemoglobin concentration.

sTfR concentrations were inversely related to hemoglobin concentrations. The geometric mean sTfR concentrations in children with hemoglobin concentrations <100, 100–110, and ≥110 g/L were 10.7, 7.5, and 6.8 mg/L, respectively (*P* < 0.0001).

Multiple regression analyses

The malaria-associated increase in sTfR concentrations did not vary by age class. The estimated geometric mean concentrations of sTfR and ferritin for each of the cross-tabulated categories of the variables malaria and hemoglobin concentration class are shown in **Figure 2**. In children without malaria or inflammation, hemoglobin concentration was inversely associ-

TABLE 2
Associations of malaria with indicators of iron status

	No malaria	Malaria	Difference	
			Estimate (95% CI)	<i>P</i>
Hemoglobin (g/L) ¹	104.1 [262]	92.7 [56]	11.3 (6.4, 16.3)	0.0001
Serum soluble transferrin receptor (mg/L) ²	7.8 [192]	11.4 [32]	3.5 (1.0, 6.7)	0.005
Ferritin (μg/L) ²	11.9 [168]	21.6 [32]	9.6 (0.1, 26.8)	0.05
C-reactive protein (mg/L) ²	6.8 [76]	12.5 [15]	5.6 (1.7, 11.5)	0.004

¹Arithmetic \bar{x} ; *n* in brackets.

²Geometric \bar{x} ; *n* in brackets.

TABLE 3Iron status of children by age, malaria status, and concentration of C-reactive protein¹

Iron status indicator and age	Prevalence (95% CI)	P
Anemia (hemoglobin <110 g/L)		
No malaria		
2–11 mo	62 (50, 73) [80]	0.01
12–23 mo	79 (70, 88) [94]	
≥24 mo	55 (44, 67) [88]	
All	66 (59, 72) [262]	
Malaria		
2–11 mo	90 ²	0.83
12–23 mo	86 ²	
≥24 mo	82 ²	
All	85 (75, 96)	
Iron deficiency (serum ferritin <12 µg/L) ³		
Excluding children with malaria		
2–11 mo	26 (12, 40) [57]	0.006
12–23 mo	63 (48, 78) [53]	
≥24 mo	47 (31, 63) [57]	
All	45 (36, 54) [168]	
Excluding children with CRP ≥8 mg/L		
2–11 mo	49 (25, 72) [19]	0.02
12–23 mo	77 (59, 96) [20]	
≥24 mo	31 (9, 54) [17]	
All	53 (39, 67) [55]	
Iron deficiency anemia (hemoglobin <110 g/L and serum ferritin <12 µg/L) ³		
Excluding children with malaria		
2–11 mo	21 (9, 33) [57]	0.005
12–23 mo	55 (40, 70) [53]	
≥24 mo	25 (13, 37) [57]	
All	33 (26, 41) [168]	
Excluding children with CRP ≥8 mg/L		
2–11 mo	38 (15, 60) [19]	0.01
12–23 mo	72 (54, 90) [20]	
≥24 mo	25 (4, 45) [17]	
All	46 (32, 59) [55]	

¹n in brackets. 95% CIs were calculated by using normal approximation. Sample sizes are based on weighted observations and are not necessarily integers; because of rounding, the summed values of the 3 age categories may not equal the total given for all ages.

²The conventional approximation using the hypergeometric distribution was not allowed because the data were clustered.

³Serum ferritin concentration reacts as an acute phase protein; this was controlled for by excluding children with malaria or with serum C-reactive protein (CRP) concentration ≥8 mg/L.

ated with sTfR concentration (Figure 2, Table 4) and positively associated with serum ferritin concentration (Figure 2). When adjusted for malaria and serum C-reactive protein concentration quartile, children with hemoglobin concentrations of 100–110 and ≥110 g/L had sTfR concentrations proportionally lower by factors of 0.77 and 0.67 than those for the reference class of children with hemoglobin concentrations <100 g/L. In these same children, serum ferritin concentrations were elevated by factors of 1.31 and 1.49, respectively (NS).

In the multiple regression models, malaria was associated with elevated serum concentrations of both sTfR and ferritin. Serum C-reactive protein concentration showed no consistent or substantial association with sTfR concentration, but serum ferritin concentrations were substantially elevated when serum C-reactive protein concentrations were ≥8.4 mg/L. Children aged 12–23 and >24 mo had serum ferritin concentrations pro-

portionally lower by factors of 0.48 and 0.72, respectively, than concentrations for the reference class of children aged 2–11 mo (NS). The geometric mean sTfR concentrations calculated on the basis of the model presented in Table 4 are shown in Table 5.

DISCUSSION

Malaria is associated with increased sTfR concentrations, both when assessed by crude analysis and when the effects of hemoglobin concentration and serum C-reactive protein concentration are considered. Malaria is also associated with decreased hemoglobin concentrations. These findings are consistent with our hypothesis that malaria-associated hemolysis results in increased erythropoiesis. The inflammatory response to malaria is reflected by increased serum concentrations of C-reactive protein and ferritin. A lack of association between serum C-reactive protein concentration and serum sTfR concentration, even after adjustment for potential confounding by malarial infection, suggests that the inflammatory response does not influence sTfR concentrations. It can therefore be safely assumed that sTfR concentrations measure both the rate of erythropoiesis and the deficit in functional iron in the erythron.

Sickle cell trait is not substantially associated with hemoglobin concentration (26) and occurs rarely in the Akamba tribe (27). Urinary schistosomiasis is seldom reported by local medical personnel, and other helminth infections were uncommon. None of these factors is likely to have substantially confounded or modified the relations observed. The prevalence of anemia

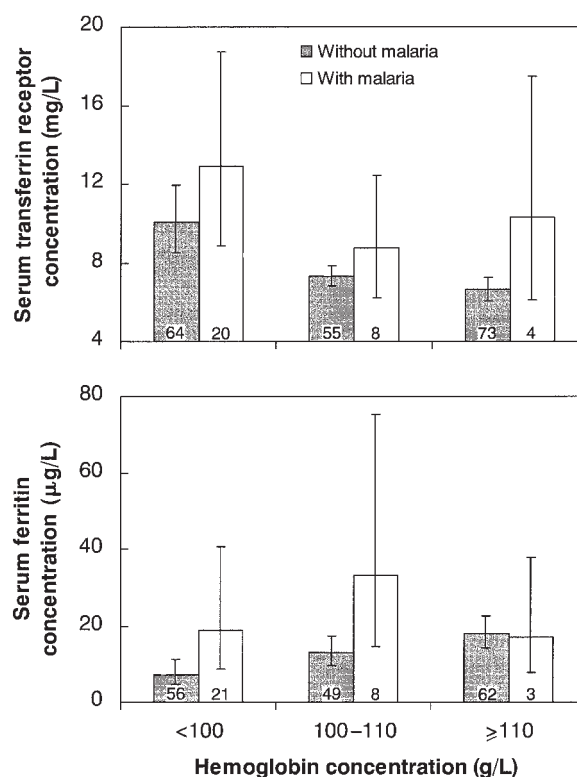


FIGURE 2. Geometric mean (and 95% CI) serum concentrations of soluble transferrin receptor and ferritin in association with hemoglobin concentrations in children without and with malaria. The numbers in the bars indicate the sample size for each category.

TABLE 4
Multiple regression model of serum concentrations of soluble transferrin receptor (model 1; $n = 88$) and ferritin (model 2; $n = 90$)¹

Variables	Factor (95% CI)	Wald <i>F</i>	df	<i>P</i>
Outcome: serum soluble transferrin receptor (mg/L)				
Hemoglobin (g/L)	—	5.3	2	0.01
<100	—			
100–110	0.77 (0.62, 0.96)			
≥110	0.67 (0.53, 0.86)			
Malaria	1.50 (1.07, 2.10)	5.6	1	0.02
Serum C-reactive protein quartile (mg/L)	—	0.2	3	0.92
<4.7	—			
4.7–6.3	1.10 (0.83, 1.44)			
6.3–8.4	1.04 (0.83, 1.32)			
≥8.4	1.00 (0.82, 1.22)			
Outcome: serum ferritin (μg/L)				
Hemoglobin concentration (g/L)	—	1.0	2	0.37
<100	—			
100–110	1.31 (0.70, 2.44)			
≥110	1.49 (0.86, 2.56)			
Malaria	2.13 (1.05, 4.30)	4.4	1	0.04
Serum C-reactive protein quartile (mg/L)	—	6.0	3	0.002
<4.7	—			
4.7 to <6.3	2.37 (0.98, 5.76)			
6.3–8.4	2.13 (0.92, 4.92)			
≥8.4	5.54 (2.33, 13.20)			
Age (mo)	—	2.2	2	0.13
2–12	—			
12–23	0.48 (0.24, 0.96)			
>24	0.72 (0.39, 1.33)			

¹Effect estimates were first obtained for the regression coefficient (β) indicating changes in the log serum soluble transferrin receptor (sTfR) concentration; the effect estimates reported here (10^β) are proportional instead of additive. Thus, factors indicate the proportional change in sTfR concentration relative to children with hemoglobin concentrations <100 g/L and without malaria, and in the lowest serum C-reactive protein concentration quartile, or the proportional change in serum ferritin concentration relative to infants with hemoglobin concentrations <100 g/L, without malaria, and in the lowest serum C-reactive protein concentration quartile.

(69%) was high but is typical for east African preschool children (28). Hemoglobin concentration and its relation to malaria were not found to be different between those children for whom data on sTfR concentration were or were not available (not shown); hence, there was no evidence for bias due to missing values. We expect similar results to be found in preschool children asymptomatic for malaria in other areas of unstable malaria.

It is often difficult to detect iron deficiency in individual children in malarious areas. Serum ferritin concentrations <12 μg/L are highly predictive of depleted iron stores (29), regardless of whether infection or inflammation is present. Serum ferritin concentrations increase rapidly during malarial infection (30–32), probably as part of a host immune response (33). This was confirmed by our results. Hence, when coupled with anemia, normal or high serum ferritin concentrations indicate infection, but this may mask concomitant iron deficiency. In these cases, contrary to the case for other infections (34), sTfR concentration is not useful in detecting iron deficiency because malaria-associated hemolysis may also increase sTfR concentration. In the absence of malaria, serum ferritin concentrations <12 mg/L often fail to detect existing iron deficiency, as indicated by higher estimated prevalences of iron deficiency in children with serum C-reactive protein concentrations <8 mg/L than in children without malaria (Table 3). This may be due to serum ferritin concentrations being increased by asymptomatic infections other than malaria and suggests that iron deficiency can be accurately diagnosed only in children with low serum C-reactive protein concentrations.

Reports by Stoltzfus et al (35, 36) suggest that malaria leads to increased serum ferritin concentrations in young children but not in older children in areas with highly endemic malaria. The latter group may have developed partial protective immunity that prevented asymptomatic malaria from affecting hemoglobin concentrations or serum ferritin concentrations.

In children without malaria or inflammation, low hemoglobin concentrations were associated with higher sTfR concentrations and lower serum ferritin concentrations (Table 4). The latter probably occurred because low hemoglobin concentrations in these children indicate iron deficiency, which develops in several stages. When iron stores are still present, iron depletion results in reduced serum ferritin concentrations and a marginal increase in sTfR concentrations. Beyond this stage, iron depletion results in a marginal reduction of serum ferritin concentrations and substantial increases in sTfR concentrations (29). Concentrations of sTfR then indicate the iron deficit in functional compartments of the body (including hemoglobin). In addition, sTfR concentrations in these children are likely to have increased as a result of increased erythropoietic activity in response to the anemia (37).

Malaria was associated with sTfR concentrations that were higher than expected for the degree of anemia (Figure 2, Tables 4 and 5). Brabin (6) speculated that malaria may induce iron deficiency through iron loss in urine after hemolysis, reduced iron absorption, or iron sequestration in macrophages of the mononuclear phagocyte system. Iron loss associated with hematuria may occur after severe hemolysis, but in mild hemolysis, free heme iron

TABLE 5

Estimated geometric mean serum concentrations of soluble transferrin receptor ($n = 224$) and ferritin ($n = 196$) based on the multiple regression models reported in Table 4

Hemoglobin (g/L)	Serum soluble transferrin receptor ¹			Serum ferritin ²		
	No malaria	Malaria	Difference	No malaria	Malaria	Difference
		<i>mg/L</i>			<i>μg/L</i>	
<100	8.9	13.3	4.4	5.5	11.6	6.2
100–110	6.8	10.2	3.4	7.1	15.2	8.1
≥110	6.0	8.9	3.0	8.1	17.3	9.1

¹For children with serum C-reactive protein concentrations <4.7, 4.7 to <6.3, 6.3–8.4, and ≥8.4 mg/L, these values must be multiplied by 1.00, 1.10, 1.04, and 1.00, respectively.

²For children with serum C-reactive protein concentrations <4.7, 4.7 to <6.3, 6.3–8.4, and ≥8.4 mg/L, these values must be multiplied by 1.00, 2.37, 2.13, and 5.54, respectively; for children aged 2–11, 12–23, and ≥24 mo, these values must also be multiplied by 1.00, 0.48, and 0.72, respectively (see Table 4).


or hemoglobin is probably recycled for use in normal body functions. In our study, children were asymptomatic or perhaps convalescing from recent malaria attacks, so that none or very few were likely to have severe hemolysis. Decreased iron absorption reportedly plays a minor role in the anemia of inflammation (38). The shift in iron distribution observed in inflammatory diseases and infections has long been believed to be a cause of reduced erythropoiesis, but this is not accompanied by an increase in sTfR concentration (15, 34). The observed increase in sTfR concentration is therefore unlikely to be due to iron deficiency. Together with our finding that malaria is associated with decreased hemoglobin concentrations (Table 2) and anemia (Table 3), our observations are consistent with the notion that malaria-induced hemolysis leads to lower hemoglobin concentrations, which in turn result in increased erythropoiesis that is appropriate for the degree of anemia.

It cannot be ruled out that, in addition, sTfR concentrations are elevated in malaria because of ineffective erythropoiesis, whereby developing erythroblasts are prematurely phagocytosed in the marrow by macrophages before they produce mature red cells. Morphologic evidence of expanded erythroid mass with ineffective erythropoiesis was described in studies of the bone marrow of patients with severe anemia and chronic falciparum malaria (39), uncomplicated falciparum malaria (32), and febrile attacks of vivax malaria (40). These studies found abnormalities in developing erythroblasts and evidence of increased phagocytosis of erythroblasts at various stages of degradation. Results of studies of cobalamin deficiency support the notion that ineffective erythropoiesis is associated with increased sTfR concentrations (41, 42). Indicators of cobalamin deficiency were not routinely studied by us, but no megalocytosis was observed. It also cannot be ruled out that sTfR concentrations are increased in persons with malaria because of iron sequestration in hemozoin. This is a waste product found in circulating or phagocytosed red cells that results from hemoglobin degradation by malaria parasites.

Several other studies support the finding of increased sTfR concentration in persons with asymptomatic malaria (36, 43, 44). Mockenhaupt et al (44) found increased sTfR concentrations in persons with asymptomatic and mildly symptomatic falciparum malaria when the analysis was adjusted for hemoglobin concentration. Stoltzfus et al (36) found that sTfR concentrations increased with parasite density in children with asymptomatic malaria but found no clear evidence for increased sTfR concentrations when they adjusted for hemoglobin concentration. In pregnant women attending antenatal clinics in Malawi, Huddle et al (45) found that malaria was associated with marginally and not significantly

increased sTfR concentrations, despite hemoglobin concentrations being considerably lower. Williams et al (46) found no significant difference in sTfR concentration and hemoglobin concentration between children in Vanuatu with asymptomatic malaria and children with no malaria.

A decrease in sTfR concentrations was found in symptomatic malaria in 2 studies (46, 47), but there was no association in 2 others (48, 49), possibly because of the small sample size in these studies. Williams et al (46) interpreted the decrease in sTfR concentrations as evidence for decreased erythropoiesis in symptomatic malaria, either as a result of acute erythropoietin deficiency (50) or suppression of marrow response to erythropoietin (51, 52). However, some studies reported erythropoiesis in symptomatic malaria to be increased, albeit ineffectively and lower than expected for the degree of anemia (32, 39, 40), whereas others reported marrow hypoplasia and decreased red cell production (53, 54). Available evidence also indicates erythropoietin concentrations in malaria to be increased (50, 52, 55–57), although not as much as expected for the degree of anemia (50, 55, 56).

In summary, asymptomatic malaria was associated with lower hemoglobin concentrations and elevated sTfR concentrations, findings consistent with the notion that malaria-induced hemolysis leads to increased erythropoiesis. Reported discrepancies about the association between malaria and sTfR concentration are likely to remain until further reports become available on changes in sTfR concentration after treatment of patients with various forms of malaria. Individuals with high concentrations of serum C-reactive protein or similar acute phase reactants should be excluded from analysis when serum ferritin concentrations <12 μg/L are used to measure the prevalence of iron deficiency in malarious areas. 

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