

Low-Dose Daily Iron Supplementation for 12 Months Does Not Increase the Prevalence of Malarial Infection or Density of Parasites in Young Zanzibari Children

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ABSTRACT Conflicting evidence exists on the possible role of iron supplementation in the predisposition to malaria infection or the enhancement of its clinical severity. Where anemia prevalence is >40%, current guidelines are to provide low-dose daily iron to young children for up to 18 mo. Earlier studies used doses higher than the current guidelines, intermittent doses, or have supplemented for durations ≤ 4 mo. We aimed to assess the effect of low-dose, long-term iron supplementation on malaria infection using a double-blind, placebo-controlled, randomized design, and to examine possible subgroup effects by season and child age. The study was conducted in Pemba Island, Zanzibar, where *Plasmodium falciparum* malaria has year-round high transmission. A community-based sample of 614 children 4–71 mo old was randomly allocated to 10 mg/d iron or placebo for 12 mo. Outcome measures were the prevalence and density of malaria infection, which was assessed by blood films at monthly intervals. At baseline, 94.4% were anemic (hemoglobin < 110 g/L), 48.1% were stunted (height-for-age Z-score less than -2) and >80% had malaria-positive blood films. No significant differences in malarionometric indices were observed between children in the iron-supplemented and placebo groups. Parasite density was higher in certain months and in younger children, but iron supplementation was not associated with any malarial infection outcome in any season or age subgroup. We conclude that in this environment of high malaria transmission, daily oral low-dose supplementation of iron for 12 mo did not affect the prevalence of malaria infection or parasite density. *J. Nutr.* 134: 3037–3041, 2004.

KEY WORDS: • malaria • iron • anemia • children • epidemiology

Malaria is a major cause of morbidity and mortality in many parts of the world. Despite intensive studies of the disease and many public health interventions over the last several decades, malaria continues to kill 1 million people each year; 700,000 of these are children < 5 y old (1). Of all malaria cases, ~90% occur in Africa (2), with southeast Asia having the next largest percentage, ~8%.

Childhood anemia is a major health problem in sub-Saharan Africa, and severe anemia from malaria is one of the leading causes of morbidity and mortality in hospitalized patients (3). Malaria and iron deficiency are known contributors to anemia, especially in preschool children and pregnant women (4). Although food fortification with iron is advocated as the ideal long-term solution to the problem of iron deficiency in children, iron supplementation is currently a recommended means of treating and preventing anemia (5). Yet, there is conflicting evidence on the effects of iron supplementation and its possible role in predisposing to malaria infection or enhancing its clinical severity (6,7). Several studies found

that supplementation with iron could increase susceptibility to malaria (8–10), whereas others found no association or marginal nonsignificant protection (11–14).

A 1999 consensus statement by the International Nutritional Anemia Consultative Group (INACG) on this topic was based on a meta-analysis of 9 published and 4 unpublished controlled randomized trials (15). Moderate increases in some malarionometric indices were noted, but there was minimal evidence for exacerbation of those indices for oral low doses of iron in preschool children. Since then, at least 3 new controlled trials were published, all reporting no adverse effects of iron (11,14,16), although one of these used twice weekly (not daily) supplementation (14) and another used a multivitamin with iron (not iron alone) (16). The role of iron supplementation in possibly increasing susceptibility to malaria is an important question with programmatic urgency. Data from the present study were included in the INACG meta-analysis (15) as one of the unpublished data sets, but without the subgroup analyses by season and age, presented here.

Trends and general patterns of malaria transmission vary greatly geographically and the interrelations among parasite,

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vector, environment, and host are very complex. The natural history of malaria is greatly influenced by the level of transmission (1). Thus, it is important to analyze the effects of iron supplementation in controlled trials, and with attention to season and level of immunity.

We aimed to determine the effects of long-term, low-dose daily iron supplementation on parasitemia in preschool children in a holoendemic area of coastal east Africa (Pemba Island, Zanzibar), and to determine also whether these effects were dependent on season of year or age subgroup.

SUBJECTS AND METHODS

Study area, climate and population. This study was carried out in Kengeja village, Pemba Island, Zanzibar in the United Republic of Tanzania between 1996 and 1997. Pemba is the smaller of the 2 main islands off the east coast of mainland Tanzania. Of the >300,000 inhabitants, 80% live in small villages and make their living from subsistence agriculture, fishing, and cultivation of cloves and seaweed for export. *Plasmodium falciparum* malaria is holoendemic with year-round transmission. The average annual rainfall in Zanzibar is 1500 mm. The "small rains" occur typically between November and December and the "big rains" between April and June. The intensity of transmission is representative of coastal east Africa and possibly many low-altitude environments of Africa with significant annual rainfall.

Study design and sample. The primary objectives of the main study were to examine alternative methods to reduce the prevalence and severity of anemia in preschool children in Pemba through deworming and iron supplementation interventions. Prospective monthly data on malaria infection permitted analysis of the dynamics of malaria transmission as a secondary aim of the larger trial.

The study was a double-blind, randomized, placebo-controlled, 2 × 2 factorial trial of daily low-dose iron supplementation and quarterly deworming treatment with single-dose mebendazole. Each child received either daily iron (10 mg/d in syrup for 12 mo) or placebo, and mebendazole (500 mg as a single dose) or placebo every 3 mo. The main study findings, including the effect on growth and on motor and language development were reported previously (17,18). The study was approved by the internal review boards of The Johns Hopkins Bloomberg School of Public Health, the WHO, and the Zanzibar Ministry of Health.

In June and July 1996, a community census was conducted in the village of Kengeja to identify age-eligible children. A database was created of all children who would be between the ages of 6 and 59 mo on September 1, 1996. The database contained 684 children from 451 households, all of whose parents gave informed consent for their children to participate in the study. Of the 684, 614 completed the baseline assessments in September 1996 and were subsequently followed for the duration of the year. Children's ages were verified at the time of a baseline examination with official documents and ranged between 4 and 71 mo.

Randomization and intervention. Randomization to iron treatment was done on the household level rather than the individual child level to minimize inadvertent crossover when there were multiple enrolled children in a household. The mother was responsible for giving the supplements. Randomization was also stratified by age to ensure that equal numbers of 2 age groups were represented in the treatment arms. Households were first grouped into 3 strata, those with children < 36 mo, those with children ≥ 36 mo, and those with 1 or more children in each age strata. Then within each of these strata, households were randomly assigned to receive iron or placebo. After randomization to iron or placebo, these children were further individually randomized to receive mebendazole or placebo. In the present paper, we report only the effect for iron supplementation. There was no reason to expect that mebendazole would have affected malaria and, indeed, no effects were observed (data not shown).

Iron supplementation was in liquid form, containing 20 g/L ferrous sulfate intended to provide the daily requirement of 10 mg of elemental iron. Both the iron and placebo were matched for color, flavor, and consistency and packaged in identical bottles with child-proof 1-mL dropper caps (both supplied by ALPharma). At baseline,

mothers were trained to administer 0.5 mL daily to each child in the study for 1 y. During the year, study staff made weekly visits to each mother to assess compliance by asking how many days in the past week was supplement given, to attempt to address any compliance issues using a problem-solving algorithm, and to provide additional supplement as needed.

Assessment methods. At baseline and at the end of the trial, venous blood samples (3 mL) were collected from all children into Vacutainer tubes (BD, Franklin Lakes) for iron status assessments. During the trial period, monthly assessments of malaria infection from finger or heel pricks were made on a 50% subsample of the 614 children enrolled to minimize undue pricks for blood. To do this, the sample was randomly divided into 2 groups, A and B. Group A was measured at month 1, or October of 1996, and reassessed on alternate months thereafter, i.e., months 3, 5, 7, 9, 11 and 12. Group B was measured at month 2 and alternate months thereafter, i.e., months 4, 6, 8, 10 and 12. All children were assessed at baseline and 12 mo.

Drops of venous or capillary whole blood were immediately used to make thin and thick films and to determine hemoglobin concentrations using a HemoCue hemoglobinometer (HemoCue, AB). Thick blood films were stained with Giemsa and the numbers of malaria parasites were counted against leukocytes. The microscopist counted fields containing >200 leukocytes. If <10 parasites were observed, the counting continued up to 500 leukocytes. Parasite densities were calculated assuming there are 8×10^9 leukocytes/L of blood (19). A random sample of 10% of the smears was reread to check for quality of readings.

Data analysis. Outcome measures for this analysis are the prevalence of malaria infection and parasite densities. Denominators used to calculate prevalence rates consisted of all children evaluated during that particular month. Parasite densities are reported as geometric means because they were skewed to higher values. Children with no parasites are included in the geometric mean calculations by setting their density value equal to 1 before making the log transformation to normalize the distribution. High parasite density was defined as ≥ 5000 parasites/ μ L of blood. This cutoff value was used because it was shown that the likelihood of a clinical attack in endemic regions increases when blood-stage density exceeds 5000 parasites/ μ L (20,21).

The study was designed to examine effects in 2 age groups; in this analysis, we used 30 mo as the age cutoff point, consistent with other papers from this trial (18). For regression analysis, age was further subdivided into 6 groups (<12, 12–23, 24–35, 36–47, 48–59, and 60–72 mo of age) and modeled as dummy variables so as to not impose an ordinal relationship. Season was also modeled as a dummy variable. The months of January–April correspond to the dry season and beginning of heavy rains, the months of May–August correspond to the end and after the long rainy season, and the months of September–December correspond to the months around the short rainy period.

Logistic regression models were used to estimate the independent influences of season, age, and iron supplementation adjusted for potential confounders on the prevalence of infection. To account for the correlation between multiple measures on each child, generalized estimating equations were used to calculate the adjusted odds ratios (OR) and their appropriate standard errors (22). Interactions of season, age, and iron supplementation were also examined. Differences were considered significant if $P < 0.05$, and are noted in the text if < 0.10 . All statistical analyses were performed using STATA, version 6.0.

RESULTS

The children in the 2 treatment arms were comparable at baseline (Table 1). Anemia was very common, with 94.4% of values < 110 g/L and 17.2% < 70 g/L. Stunting was very prevalent (height-for-age Z-score less than -2.0, 48.1%) but wasting was not (weight-for-height Z-score less than -2.0, 4.8%). Over 80% of the children in the 2 groups were infected with the *P. falciparum* parasite and the distribution of children within the 3 strata of parasite density did not differ in the 2 groups at baseline.

TABLE 1

Baseline characteristics of 4- to 71-mo-old Zanzibari children by treatment group¹

	Iron group (n = 307)	Placebo group (n = 307)	P-value ²
Age, mo	33.7 ± 16.2 ²	33.0 ± 17.1	0.57
Sex, male	164 (53.4) ³	159 (51.8)	0.69
Currently breast-fed	81 (26.4)	98 (32.0)	0.13
Height-for-age Z-score	-1.76 ± 1.25	-1.67 ± 1.17	0.32
Weight-for-height Z-score	-0.66 ± 0.77	-0.67 ± 0.89	0.96
Hemoglobin, g/L	87 ± 15	86 ± 16	0.57
Malaria positive	253 (82.4)	261 (85.0)	0.38
Parasite density, n/μL			0.63
0	54 (17.6)	46 (15.0)	
1-4999	215 (70.0)	225 (73.3)	
≥5000	38 (12.4)	36 (11.7)	

¹ Values are means ± SD or n (%).

² P-values for continuous variables were calculated using Student's two-sided t test. P-values for categorical variables were calculated using Pearson's χ^2 .

At the end of the 12 mo, 538 (87.6%) children had completed the trial, 273 in the iron group and 265 in the placebo group (Fig. 1). The supplement was well tolerated by the children and according to the mothers' weekly report, >90% of the children received at least 70% of the intended doses. As

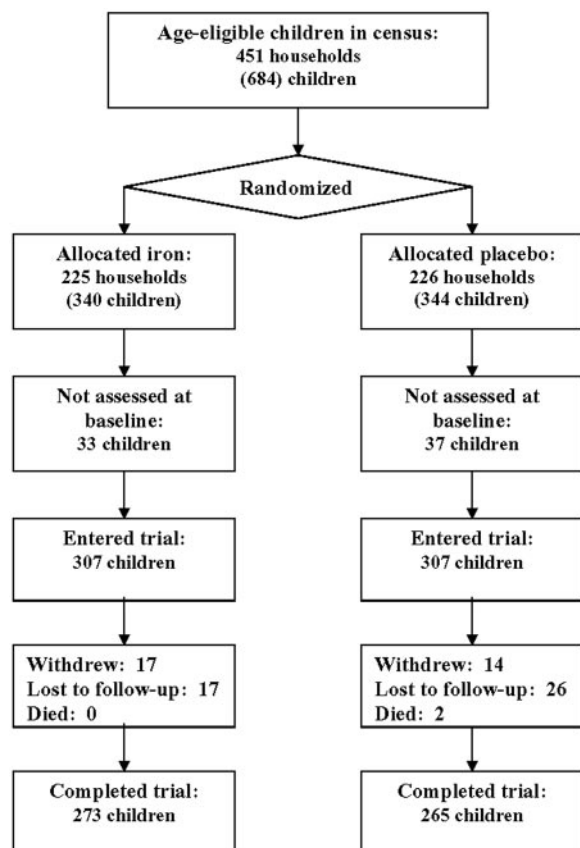


FIGURE 1 Trial profile.

reported elsewhere (18), at 12 mo, the iron-supplemented group had significantly higher serum ferritin (geometric mean: 55.4 vs. 40.7 mg/L, $P < 0.001$) and significantly lower erythrocyte protoporphyrin (mean ± SD: 84 ± 68 vs. 106 ± 92 μmol/mol heme, $P < 0.005$), compared with the placebo group.

The prevalence of infection was fairly constant throughout the year with no distinct evidence of seasonality (data not shown). Infection rates ranged from 71.8% in the month of January 1998 to 88% in August 1998. In contrast, there was pronounced seasonality in the density of the infection (Fig. 2). Geometric mean parasite density (GMPD) for all of the children in the study was 5.4 times higher in the month of August (752 parasites/μL), after the long rains, than in January (138 parasites/μL, $P < 0.0001$), in the dry season.

Iron effect on malaria infection. No significant differences in malarimetric indices were observed between children in the iron and placebo groups. GMPDs of the 2 treatment groups were similar for almost every month of the year (Fig. 2) with the exception of December and August when the placebo group had marginally higher GMPD than the iron group ($P = 0.04$ and $P = 0.08$, respectively). Rates of high-density parasitemia (>5000 parasites/μL) showed a similar seasonal pattern and also did not differ between the iron or placebo group in any month of the year (data not shown).

Malarial infection was associated with age, and was less dense in older children. However, iron treatment was not associated with any difference in malarimetric indices when examined by age subgroup. In children < 30 mo old, the GMPD was 3402 parasites/μL in the iron group and 3422 parasites/μL in the placebo group ($P = 0.95$); in children ≥ 30 mo, the GMPD was 2188 parasites/μL in the iron group and 2046 parasites/μL in the placebo group ($P = 0.49$). We further defined as an outcome any occurrence of parasite density > 5000 at any time during the year. In children < 30 mo, 47.8% in the iron group and 52.2% in the placebo group experienced high parasitemia by this criterion ($P = 0.40$). In children ≥ 30 mo, 50.0% in the iron group and 50.0% in the placebo group had high parasitemia at any time ($P = 1.00$).

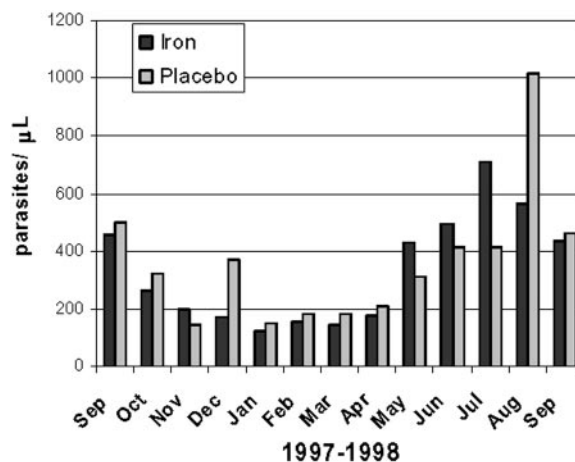


FIGURE 2 Geometric mean parasite density in Zanzibari children by calendar month and treatment group. Treatments were 10 mg/d oral iron or placebo. In the months October 1997 to August 1998, malarimetry was carried out in a 50% random subsample. The numbers of observations/mo as shown from left to right are: 614, 287, 271, 274, 280, 224, 278, 260, 277, 252, 275, 267, 536. Comparisons between treatment groups in each month are all nonsignificant ($P > 0.05$) except in December 1997, $P = 0.04$.

Analyses using multiple logistic regression models show the simultaneous effects of season, age, and iron on malaria outcomes (Table 2). In this model, both infection prevalence and high-density infections showed seasonal increases in May–August, but seasonality was much more marked for high-density parasitemia. These analyses confirmed that iron supplementation was not associated with increased risk of malaria infection or of high-density parasitemia. The probability of infection at any time was 10% less for the iron group, but this difference was nonsignificant (OR = 0.90, 95% CI: 0.72–1.14). The odds of high-density parasitemia were 4% greater for the iron group, but were not significantly different from unity (OR = 1.04, 95% CI: 0.82–1.34).

The regression results provide clear evidence that as the children aged, both exposure to malaria infection and immunity to the infection increased. The odds of being slide-positive at any point in time throughout the year was 61% greater for 2-y olds (OR = 1.61, 95% CI: 0.98–2.63) than for children 4–11 mo old, and the OR steadily increased to 2.44 (95% CI: 1.40–4.27) for 5-y olds (Table 2). However, as the children aged, their ability to limit the density of infection became more evident. The odds of having high-density parasitemia increased (nonsignificantly) for 2-y olds vs. 4- to 11-mo olds, but then decreased steadily with age.

Interactions between season and age, season and iron supplementation, and age and iron supplementation were examined in the multivariate models. No significant effect modifications were found.

DISCUSSION

The main finding of this trial was that low-dose oral supplementation with iron for a period of 12 mo did not increase malarial infection in the children of our study. No effect of iron was observed on either prevalence of infection or density of parasites. This result seems to agree with other

studies that found that iron did not affect susceptibility to malaria (11–14,16)

The possibility was raised that with long durations, iron supplementation would increase malaria morbidity even if it did not do so in the short term (15). To our knowledge, there are no other published studies examining the influence of longer-term daily supplementation (i.e., >4 mo). Our study was unique in that it was able to show that there is no evidence to support the hypothesis that long-term iron supplementation would increase the risk of malaria infection. Also, there was no evidence that iron supplementation is more deleterious in high-transmission seasons (i.e., July–September) in Pemba.

This is an important finding because iron supplementation in high-risk young children is urgently needed to combat the severe anemia that threatens children's development (17) and possibly their survival (23). In addition, HIV risk is high in sub-Saharan Africa and overlaps areas in which there is high malaria transmission. In the face of the HIV epidemic and other blood-borne infections, it is important to minimize unnecessary blood transfusions used to treat severe anemia (24). Blood transfusion to combat life-threatening severe anemia is a common practice in sub-Saharan Africa (1), and iron supplementation to improve the hematological status of children in malaria-endemic areas of sub-Saharan Africa may an important means to potentially prevent transfusions (12).

However, this study did not assess clinical malarial outcomes, and its conclusions should not be overextended. Malaria was not a primary outcome of this study and the sample size was likely too small to obtain significant findings for clinical outcomes. The number of studies that have assessed clinical malaria attacks in children undergoing iron supplementation is rather small [$n = 8$ in the 1999 INACG meta-analysis (15)], and clinical malaria was variously defined in these studies, often with low specificity. As stated by INACG, "clinically important risk elevations are not ruled out by these data." Parasite count in the peripheral blood can change within an individual in a given day and from day to day (25,26), and the progression from infection to clinical episode is not well understood (27).

In Pemba, we found a consistently high prevalence of infection across all months of the study, but there was striking seasonality in the density of parasitemia. The geometric mean parasite density of all children in the month of August, after the long rainy season, was >5 times that found in the dry season. This could be due to increased numbers of infective mosquitoes during and after the long rainy season. Shiff et al. (28) found seasonal patterns of anopheline abundance in villages surrounding coastal holoendemic Bagamoyo, Tanzania, an area with malaria transmission and precipitation patterns similar to those of Pemba, Zanzibar. They found that the greatest numbers of infective mosquitoes occurred during the months of June–August and that numbers of infective mosquitoes and infected mosquito bite rates were at the lowest in the dry months of January–March. In Pemba, a seasonal peak in malaria morbidity that follows the heavy rains (i.e., July–August) was observed in health facilities and confirmed by local health statistics.

In summary, our data provide strong evidence that in a holoendemic transmission area, the prevalence of infection and the age-related decline in the density of infection are not adversely affected by long-term, low-dose iron supplementation. No adverse effect on these infection parameters was detected in the most vulnerable age groups, or during the most high-risk months of the year.

It is noteworthy that the supplementation was low dose,

TABLE 2

Adjusted odds ratios for malaria infection and high-density parasitemia in 4- to 71-mo-old Zanzibari children over the 12-mo trial period

	Outcome variable	
	Malaria infection	≥5000 parasites/μL
Supplementation		
Placebo	1.00	1.00
Iron	0.90 (0.72–1.14) ¹	1.04 (0.82–1.34) ¹
Season		
Jan–Apr	1.00	1.00
Sept–Dec	1.34 (1.12–1.60)	1.92 (1.41–2.64)
May–Aug	1.65 (1.34–2.05)	3.80 (2.77–5.20)
Age, mo		
4–11	1.00	1.00
12–23	1.61 (0.98–2.63)	1.38 (0.70–2.75)
24–35	1.99 (1.18–3.36)	1.07 (0.52–2.22)
36–47	2.16 (1.28–3.64)	0.64 (0.30–1.35)
48–59	2.71 (1.57–4.66)	0.44 (0.20–0.94)
60–71	2.44 (1.40–4.27)	0.28 (0.12–0.64)
Sex		
Male	1.00	1.00
Female	0.90 (0.72–1.15)	0.89 (0.70–1.14)

¹ Based on 3370 observations in 614 children. Odds ratios (95% CI) were derived using generalized estimating equations that adjust for the repeated measures among the children.

that the supplement did not include folic acid, the children in this study were nearly all anemic (94% prevalence, by WHO criteria), and iron deficiency was prevalent (29). Different results might be found with higher iron dosage, in multiple micronutrient combinations, or in populations in which the prevalence of iron sufficiency is much higher.

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