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Original Article

The effect of iron versus iron plus zinc supplementation in children with malaria

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ABSTRACT

Introduction Little is known about the potential interaction of iron and zinc given to increase hemoglobin and serum ferritin in children with malaria.

Objective To study the effect of iron compared with a combination of iron and zinc supplementation on children with falciparum malaria.

Method Children with positive *Plasmodium falciparum* (n=86) were randomly assigned to a daily supplementation of 6 mg iron/kg per day plus placebo or plus 10 mg zinc per day for 30 days. All children were treated with the same regimen for the treatment of *P. falciparum*. Venous blood samples were collected at the start and end of the study. After 30 days of supplementation, the baseline and follow-up blood samples were analyzed.

Results The increase of hemoglobin concentration in the iron plus placebo group was 0.58 g/dl, while in the iron plus zinc group was 0.09 g/dl (P<0.05). Serum ferritin concentration was high in both groups before trial, yet there was no significant difference after iron supplementation.

Conclusions Iron supplementation showed significant increase in hemoglobin concentration in children with positive *P. falciparum* treated with the same regimen of treatment. Supplementation of iron alone as well as iron plus zinc had been proven ineffective to iincrease serum ferritin in children with malaria. **[Pediatr Indones 2006;46:7-12].**

Keywords: *iron, zinc, Plasmodium falciparum, hemoglobin, serum ferritin*

Endemic malaria and iron deficiency anemia coexist with the highest mortality in young children and pregnant women.[1] Children less than five years old in malaria endemic areas are at risk of protein-energy malnutrition, as well as deficiencies in micronutrients including zinc.[2]

Iron deficiency is grouped into three stages of severity. The first phase is iron depletion, where total body iron decreases; the second phase, iron-deficient erythropoiesis; and last, the most severe phase, iron deficiency anemia.[3]

There are evidence that persistent or recurrent parasitemia induces iron deficiency, although the mechanisms are uncertain. These include reduced absorption of iron during the acute period of the illness, low haptoglobin levels resulted from intravascular hemolysis will reduce the formation of haptoglobin/hemoglobin complexes which are removed from the circulation by the liver, reducing iron availability, and immobilization of iron in hemazoin complexes (malaria pigment).[4]

High intake of zinc may interfere with integration of iron into, or release from ferritin, or may result in the decrease of life span of the erythrocyte.[5] Normally, a trace of zinc rather than iron is incorporated into protoporphyrin during the final step of heme biosynthesis.[6] In iron deficiency, pro-

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toporphyrin combined with zinc to form free erythrocyte zinc protoporphyrin (ZPP), which are stable and persists throughout the life-span of the red cell.[7]

Verhoef *et al*[8] concluded that iron supplementation gives substantial health benefits that may outweigh the associated risks of adverse effects caused by malaria. Lind *et al*,[9] in a study in Central Java children, found that the effect of combined iron and zinc supplement is less efficacious than single iron supplement in improving iron and zinc status.

In the present study, we investigated the effect of zinc to increase iron absorption in children with treated falciparum malaria.

Methods

A randomized placebo-controlled clinical trial study was conducted for 30 days at Panyabungan Jae and Siabu District, a malaria endemic area in Kabupaten Mandailing Natal on October 2004. Children less than 15 years who visited the health centers, were screened. A finger stick blood sample was examined for *Plasmodium falciparum,* and those who were positive were recruited for the study. Exclusion criteria was severe malaria, defined by the presence of cerebral malaria, severe anemia, hypoglycemia, shock, spontaneous bleeding, or repeated convulsions. Prior approval was obtained from the Ethical Committee of Adam Malik Hospital, Medan.

The children were randomly assigned to one of the two treatment groups: iron plus placebo or iron plus zinc. Iron was administered as ferrous sulfate syrup containing 6 mg elemental iron/kg daily (ferrous sulfate 26.25 mg/5ml, Iberet, Abbott Indonesia). Zinc was given every day as a capsule which contained 10 mg zinc sulfate (Zinc elemental 10 mg/capsule, Novell Indonesia). Zinc and placebo capsules were indistinguishable in appearance and taste. All children were treated for malaria with chloroquine base 10 mg/kg at the first day, followed by 5 mg/kg eight hours later, and then continued with 5 mg/kg every day for the second and third day.

Three milliliters venous blood was collected before and after 30 day-supplementation period and examined for hemoglobin, hematocrite, erythrocyte, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) by an autoanalyzer (ABX Mikros 60, France), while serum ferritin was measured by immunoassay (Vidas, Biomeureux).

We also assessed body weight with MIC weighing-machine (sensitivity 0.5 kg) and body height with MIC (sensitivity 0.5 cm). Nutritional status was measured with standardized anthropometry according to CDC NCHS-WHO 2000.[10]

Sample size was calculated with hypothesis for two proportions formula and we found the minimum sample was 36 children for each group. Subjects were collected with consecutive sampling and SPSS was used for all statistical computations. We analysed the different characteristics of laboratory data between the two groups using T independent test, while nutritional status, age, and blood smear using Pearson chi square. Significance level was set at $P<0.05$.

Results

Eighty six eligible children were randomly assigned to the two treatment groups, 44 for iron plus placebo group and 42 for iron plus zinc group. After supplementation, 69 children (80%) completed the four-week-course (**Figure 1**). Distribution of baseline characteristics in the intervention groups were similar (**Table 1**).

After supplementation, the increase of hemoglobin, hematocrit, and MCV was significant in the iron plus placebo group, while the increase of erythrocyte and MCV was significant in the iron plus zinc group (**Table 2**). The difference of delta hemoglobin concentration between the two groups was significant (**Table 3**).

Of 86 children with positive *P. falciparum* recruited, only 69 completed the study, i.e. 36 for iron plus placebo and 33 for iron plus zinc group. Only six children from iron plus placebo group and six children from iron plus zinc group remained to have *P. falciparum* after 30 days of supplementation (**Table 4**).

Discussion

Infections lead to growth faltering and malnutrition due to anorexia, loss of nutrients, malabsorbtion,

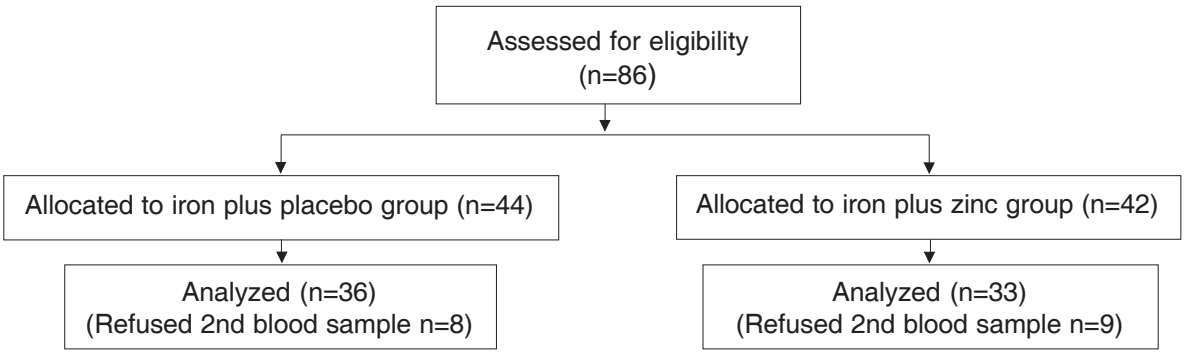

FIGURE 1. STUDY PROFILE

TABLE 1. BASELINE CHARACTERISTICS OF SUBJECTS

Characteristics	Iron plus placebo n (%)	Iron plus zinc n (%)
Age (year)		
<5	16 (36.4)	15 (35.7)
5-<10	15 (34.1)	13 (31.0)
10-<15	13 (29.5)	14 (33.3)
Sex		
Boy	26 (59.1)	19 (45.2)
Girl	18 (40.9)	23 (54.8)
Nutritional Status		
Severe Malnutrition	1 (2.3)	3 (7.1)
Moderate Malnutrition	4 (9.1)	9 (21.4)
Mild Malnutrition	9 (20.5)	1 (2.4)
Normal	24 (54.5)	22 (2.4)
Overweight	6 (13.6)	7 (4.2)

TABLE 2. LABORATORY RESULTS ON DAY 0 AND DAY 30 AFTER SUPPLEMENTATION

	Iron plus placebo (n=36)				Iron plus placebo (n=33)			
	D_0		D_{30}	P	D_0		D_{30}	P
	Mean	(SD)	Mean (SD)		Mean	(SD)	Mean (SD)	
Hemoglobin (g/dl)	11.4	(0.9)	11.9 (1.0)	0.00	11.5	(1.0)	11.6 (0.9)	0.55
Hematocrit (%)	33.9	(2.5)	35.4 (2.9)	0.00	33.5	(6.0)	34.5 (2.5)	0.31
Erythrocyte (million/mm^3)	4.7	(0.3)	4.7 (0.3)	0.34	4.6	(0.4)	4.5 (0.4)	0.03
MCV (fl)	71.8	(5.6)	75.8 (4.6)	0.00	74.4	(5.5)	77.2 (5.7)	0.00
MCHC (g/dl)	33.3	(1.7)	33.8 (0.7)	0.07	33.5	(0.9)	33.8 (1.0)	0.13
Serum feritin (ng/ml)	80.3	(67.6)	61.4(62.3)	0.20	212.3	(309.8)	97.9 (69.3)	0.03

TABLE 3. COMPARISON IN LABORATORY DIFFERENCES BETWEEN THE TWO GROUPS AFTER INTERVENTION

	Iron plus placebo (n=36) Δ Mean (SD)	Iron plus zinc (n=33) Δ Mean (SD)	P
Hemoglobin (g/dl)	0.58 (0.9)	0.09 (0.8)	0.03
Hematocrit (%)	1.43 (2.4)	10.10 (51.6)	0.32
Erythrocyte (million/mm^3)	-0.05 (0.3)	-0.14 (0.3)	0.30
MCV (fl)	4.00 (3.8)	2.76 (1.9)	0.10
MCHC (g/dl)	0.52 (1.7)	0.27 (1.0)	0.46
Serum Ferritin (ng/ml)	-18.91 (83.3)	-114.46 (269.7)	0.08

TABLE 4. BLOOD SMEAR AFTER 30 DAYS OF SUPPLEMENTATION

Plasmodium falciparum	Iron plus placebo n (%)	Iron plus zinc n (%)	Total n (%)
Negative	30 (83.3)	27 (81.8)	57 (82.6)
Positive	6 (17.4)	6 (18.2)	12 (17.4)
Total	36 (100.0)	33 (100.0)	69 (100.0)

P=0.87

changes in metabolism, and changes in feeding practices. Conversely, protein-energy malnutrition and deficiency in micronutrients, such as iron, vitamin A, and zinc are known to adversely affect immunity.[11]

According to Shankar *et al,*[12] several cross-sectional surveys showed a synergistic relationship between malnutrition and malaria. Study in Malawi, Zambia, Papua New Guinea, Sudan, Tanzania, Chad, and Zaire indicated greater risk for infection, malaria illness, or spleen enlargement among malnourished children.[12] Most children in our study had normal nutritional status.

In children with low 'normal' hemoglobin (Hb 11.0-11.4 g/dl), 28% showed a therapeutic response to iron with hemoglobin increasing 1.0 g/dl or more.[13]

When the hemoglobin or the hematocrit concentration is low, iron-responsive anemia should be considered. Low or normal MCV and/or MCH in conjunction with anemia increase the possibility of iron deficiency,[14] provided that infection, chronic inflammatory disease, thalassemia mayor, and lead poisoning have been excluded.[15] In this study, only MCV increased significantly after supplementation in both groups.

Our data suggest that asymptomatic malarial infection does not influence hemoglobin concentration, since our baseline data showed that the mean hemoglobin and mean serum ferritin were within normal limits. Infection and inflammation produce the defect known as 'mucosal block', causing an increased rate of ferritin synthesis, reflected by elevated serum ferritin.[15] Malarial infection caused a fall in hemoglobin concentration mimicking iron deficiency, but ferritin is elevated. Stoltzfus *et al,*[16] from a study in Zanzibar concluded that although ferritin values increased slightly with density >1000 parasites/μl, the values were still below the values in acute malaria. Ninety days after initiation of treatment, all values had decreased. In the beginning of our study, serum ferritin was high in the two groups, then decreased after 30 days of supplementation but still in the normal range. These might be caused by initiation of treatment with chloroquin for children with positive *Plasmodium falciparum*.

It is the single major mechanism responsible for anemia associated with chronic infectious and inflammatory disorder. These typical features offer important clues to the pathogenesis of the disorder: mild to moderate anemia, slightly decreased red blood cell survival, increased serum iron and iron-binding capacity, increased tissue iron stores and serum ferritin, decreased marrow sideroblasts, increased free erythrocyte protoporphyrin, and relative bone marrow unresponsiveness.[17]

ZPP is a normal metabolite that is formed in trace amounts during heme biosynthesis. The final reaction in the biosynthetic pathway of heme is the chelation of iron with protoporphyrin. During periods of iron insufficiency due to impaired iron utilization, zinc becomes an alternative metal substrate for ferrochelatase, which leads to an increase in ZPP formation.[18]

A study by Lind *et al*,[9] summarized that combined iron and zinc supplementation is not optimal. The combined supplement showed no effect on hemoglobin, whereas the single iron supplement had a clinically and statistically significant effect.[9] In this study the effect of zinc was also insignificant. This might be caused by the fact that since baseline data was still in normal range, the addition of zinc to the iron supplement became ineffective.

In view of the clearly demonstrated improvement in hematologic status after implementation of oral iron supplement, iron supplement programs in malaria endemic areas should be actively promoted.[19] Yulizar[20] found that the hemogram of children with falciparum malaria in the same area significantly increased after four weeks of iron supplementation.

Iron is usually given as ferrous sulphate tablets, which are relatively low-cost and easy to be transported and stored. Liquid form of iron may be more appropriate for children under two years because it is easier to be administered and may give fewer adverse gastrointestinal effects.[11] In this study, we also gave ferrous sulphate syrup for all children.

When serum ferritin concentration falls below 50 μg/l, the initiation of iron prophylaxis is appropriate. However, as long as the values are higher than 100 μg/l, iron treatment is unnecessary.[14] It is therefore important to measure serum ferritin concentrations in malaria endemic areas before iron supplementation to avoid iron overload, but the limitations are the cost and laboratory availability. Based on our study, we suggest that iron supplementation as a prophylaxis for iron deficiency anemia caused by malaria in asymptomatic children with normal or high serum ferritin concentration is unnecessary.

To summarize, Iron supplementation showed significant increase in hemoglobin concentration in children with positive *P. falciparum* treated with the same regimen of treatment. Supplementation of iron alone as well as iron plus zinc had been proven ineffective to increase serum ferritin in children with malaria.

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