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Original Article

Effect of iron and zinc supplementation in the treatment of malaria in children

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Abstract

Background Iron and zinc administration for children with malaria in endemic area were known to decrease parasitemia but data on their effectiveness when given together to increase reticulocytes as erythropoiesis parameter and hemoglobin is insufficient.

Objective To determine the effect of zinc to increase iron absorption in the treatment of *Plasmodium falciparum* malaria in children.

Methods Children with positive *Plasmodium falciparum* on their blood smear (n=86) examination were randomly assigned to daily supplementation of iron 6 mg per kg body weight per day plus placebo or iron plus zinc 10 mg per day for 30 days. Venous blood specimens were collected at the start and at the end of the study.

Results Sixty-nine children completed the supplementations and had both baseline and follow-up blood specimen study. After 30-day supplementation, the iron plus placebo and iron plus zinc groups showed significant difference on hemoglobin concentration (0.58 and 0.09 g/dl; $P<0.05$). There was no significant difference in reticulocyte production index and reticulocyte count before and after intervention in both groups. There was only significant difference in red blood cells concentration after supplementation of iron plus placebo and iron plus zinc (4.7 in 4.5 million/µl; $P<0.05$).

Conclusions Iron supplementation with or without zinc shows significant increase of hemoglobin concentration. It is slightly higher in iron plus placebo group. **[Paediatr Indones 2007; 47:256-260]**.

Keywords: *reticulocyte, iron, zinc, malaria, Plasmodium falciparum*

Iron deficiency, probably the commonest type of malnutrition in the world, affects more than half of the women and children in developing countries. The effect of malaria on iron metabolism is not fully understood, but distinct changes in iron metabolism occur during malaria and affect iron status. Malaria causes destruction of red blood cells, suppresses erythropoiesis, and results in a profound anemia.[1]

Iron deficiency, defined as diminished total body iron content, has been grouped to three stages of severity. A negative iron balance leads first to iron depletion, showed by decrease of total body iron without affecting hemoglobin synthesis. This stage is characterized by decrease in the concentration of serum ferritin which reflects the decline concentration of iron stages in the liver, spleen and bone marrow. The second stage consists of a decrease in iron transportation. This is characterized by decline concentration of serum iron and an increase in the

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iron binding capacity. These result in a decrease of transferrin saturation. The third stage is characterized by elevation of EP and gradual development of anemia with low MCV and MCH.[2] Iron is essential element of hemoglobin, myoglobin, and cytochromes, but it is also found in non-heme proteins.[3]

Iron deficiency anemia in patients with malaria increases mortality, especially in young children and pregnant women. Many clinical studies of iron deficiency report that iron repletion has protective effect on having severe and exacerbation malaria.[4] Children younger than 5 years old in malaria endemic areas are at risk of protein energy malnutrition as well as deficiency in micronutrients including zinc. Zinc supplementation in young children has been associated with reduction in the incidence and severity of diarrhea, acute respiratory infection, and malaria.[5]

There is some evidence that persistent and recurrent parasitemia induce iron deficiency, although the mechanism is uncertain. It is assumed that there is reduced absorption of iron during acute period of the illness and low haptoglobin levels resulted from intravascular hemolysis which will reduce the formation of haptoglobin/hemoglobin complexes. Then they are removed from the circulation by the liver so that iron availability decrease. There is also immobilization of iron in hemazoin complexes (malaria pigment).[6] High intake of zinc may disturb incorporation of iron into ferritin. It may also decrease life span of erythrocyte.[7] Normally, a trace of zinc rather than iron is incorporated into protoporphyrin during the final step of heme biosynthesis.[8] In iron deficiency, protoporphyrin cannot bind with iron to form heme. In the absence of iron, protoporphyrin binds with zinc to form ZnPP, which is stable and persist throughout the life-span of red cell.[9] Lind *et al*[10] in a study on children of Central Java, found that a combined supplement is less efficacious than a single one in improving iron and zinc status. The objective of this study was to investigate the effect of zinc to increase iron absorption in children with treated falciparum malaria.

Methods

This randomized placebo-controlled clinical trial was conducted in October 2004 for 30 days in Panyabungan Jae and Siabu districts, a malaria endemic area in Kabupaten Mandailing Natal. All children aged less than 15 years who came to health center with fever were screened for the study. A finger stick blood sample was taken. Children with positive *Plasmodium falciparum* in a blood smear were considered eligible for the study. Exclusion criteria included severe malaria as defined by the presence any of the followings: cerebral malaria, severe anemia, hypoglycemia, shock, spontaneous bleeding, or repeated convulsions, and if the parents refused to participate.

Children were randomly assigned to one of two treatment group: iron plus placebo or iron plus zinc. Iron was administered every day as ferrous sulfate at a target dose of 6 mg elemental iron per kg body weight daily (ferrous sulfate 26.25 mg/5 ml, Iberet 500, Abbott Indonesia). Zinc was administered every day as capsule which contains 10 mg zinc sulfate (Zinc elemental 10 mg/capsule, Novell Indonesia). Both zinc and placebo capsules were indistinguishable in taste and appearance. All children were treated with chloroquine base 10 mg/kg at the first day followed by 5 mg per kg body weight eight hours later and then continued with 5 mg per kg body weight every day on day two and three.

Venous blood was collected from the subjects before and 30 days after the supplementation period to assess hemoglobin, hematocrite, erythrocyte, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), reticulocyte count, reticulocyte production index and serum ferritin. Hemoglobin concentration was measured by photometer and serum ferritin concentrations were measured by immunoassay. We also assessed body weight and height with MIC weighing-machine (with sensitivity 0.5 kg and 0.5 cm respectively). Nutritional status was measured with standardized anthropometry according to CDC NCHS-WHO 2000.[10] Informed consent was obtained from the parents.

The minimum sample size was 36 children for each group and we used SPSS for WINDOWS 10 (SPSS Inc, Chicago) for all statistical computations. We analyzed numerical outcomes using independent t test.

Results

Eighty six eligible children were randomly assigned into two treatment groups, 44 children for iron plus

placebo group and 42 children for iron plus zinc group. Both treatment groups have similar baseline characteristics (**Tables 1** and **2**).

Out of 86 children only 69 (80%) completed the four week course of supplementation, i.e., 36 children from iron plus placebo group and 33 children from iron plus zinc group (**Figure 1**). Only six children from iron plus placebo group and six children from iron plus zinc group still had *Plasmodium falciparum* at their blood smear after 30 days of supplementation.

Tabel 3 depicts the laboratory results in the 2 groups after intervention. It shows that the only significant difference was in red blood cell count.

At day 30 the difference on hemoglobin concentration between the two groups was significant (**Table 4**).

Discussion

Iron supplementation is recommended for children at high risk of anemia, but its benefits may not outweigh the associated risk of malaria in areas of seasonal transmission. Iron supplementation gives substantial health benefits, which may outweigh possible inherent risks caused by malaria.[11]

Infections and inadequate supply of nutrients may initiate a vicious circle of deteriorating health. Infections lead to growth faltering and malnutrition by causing anorexia, loss of nutrients, changes in metabolism and malabsorption, 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.[12]

Table 1. Baseline characteristics of the participating children

Characteristics	Iron plus placebo (n=44)	Iron plus zinc (n=42)
Age (n %)		
<5	16 (36)	15 (36)
5 -	15 (34)	13 (31)
10 - 15	13 (30)	14 (33)
Sex (n %)		
Boy	26 (59)	19 (45)
Girl	18 (41)	23 (55)
Nutritional Status (n %)		
Severe Malnutrition	1 (2)	3 (7)
Moderate Malnutrition	4 (9)	9 (21)
Mild Malnutrition	9 (21)	1 (2)
Normal	24 (55)	22 (2)
Overweight	6 (14)	7 (4)

Table 2. Baseline characteristics of the laboratory data

	Iron plus placebo Mean (SD)	Iron plus zinc Mean (SD)
Hemoglobin (g/dl)	11.3 (1.0)	11.4 (1.1)
Hematocrit (%)	33.9 (2.9)	33.5 (5.6)
Erythrocyte (million/mm^3)	4.7 (0.4)	4.6 (0.4)
MCV (fl)	71.7 (4.9)	73.4 (6.4)
MCHC (g/dl)	33.2 (1.6)	33.5 (1.1)
Reticulocyte (%)	0.9 (0.5)	0.9 (0.5)

Table 3. Effect of interventions on laboratory measurement

	Iron plus placebo (n=36) Mean (SD)	Iron plus zinc (n=33) Mean (SD)	P
Hemoglobin (g/dl)	11.9 (1.0)	11.6 (0.9)	0.181
Hematocrit (%)	35.4 (2.9)	34.6 (2.5)	0.175
Erythrocyte (million/mm^3)	4.7 (0.3)	4.5 (0.4)	0.037*
MCV (fl)	75.8 (4.6)	77.2 (4.7)	0.256
MCHC (g/dl)	33.8 (0.7)	33.8 (1.0)	0.932
Reticulocyte (%)	1.2 (0.5)	0.9 (0.5)	0.147
Reticulocyte Production Index	0.57 (0.1)	0.59 (0.1)	0.060
Reticulocyte Count	0.37 (0.2)	0.46 (0.2)	0.058

* P<0.05

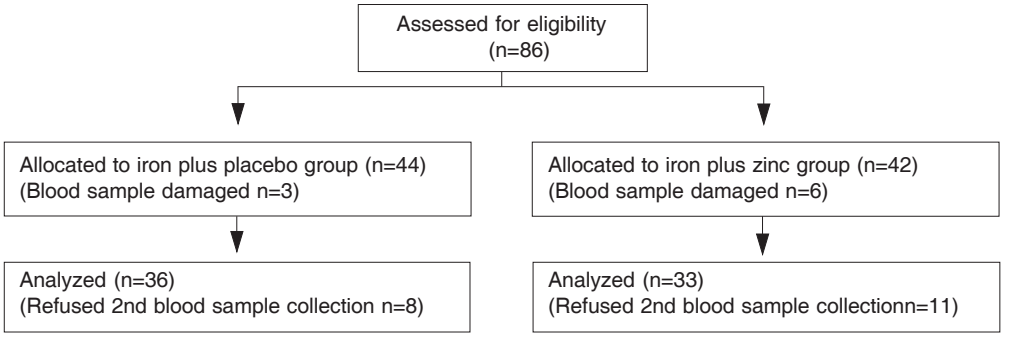

Figure 1. Trial Profile

Table 4. Difference on laboratory data after intervention

	Iron plus placebo (n=36) Mean (SD)	Iron plus zinc (n=33) Mean (SD)	P
Hemoglobin (g/dl)	0.58 (0.9)	0.09 (0.8)	0.025
Hematocrit (%)	1.43 (2.4)	10.10 (51.6)	0.316
Erythrocyte (million/mm^3)	-0.05 (0.3)	-0.14 (0.3)	0.303
MCV (fl)	4.00 (3.8)	2.76 (1.9)	0.096
MCHC (g/dl)	0.52 (1.7)	0.27 (1.0)	0.459
Reticulocyte (%)	0.26 (0.5)	0.14 (0.7)	0.421
Reticulocyte production Index	0.65 (0.15)	0.76 (0.1)	0.072
Reticulocyte count	0.76 (0.15)	0.88 (0.2)	0.081

According to Shankar *et al*[13] several cross-sectional surveys also favor 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. Most of children in our study had normal nutritional status as express by anthropometry at baseline.

Erythropoiesis is the continuous process whereby primitive erythroid progenitor cells proliferate and differentiate into mature, circulating red cells.[11] If anemia occurs, erythropoietin production increases and it stimulates erythropoiesis. One of the ways is by releasing reticulocyte from the bone marrow to the peripheral blood earlier than normal condition.[14] An increase in circulating reticulocytes is the most reliable signs of accelerated erythrocyte production.[15]

Malaria causes a shift of iron distribution from functional toward storage compartments. Serum iron concentration, iron-binding capacity and serum transferrin saturation all decreases, while serum ferritin concentrations increases. Reticulocyte count is normal or increased, but it is inappropriately low for the degree of anemia.[12] After the start of therapy, reticulocyte response may be observed within 48 to 72 hours, with a maximal response on the fifth to tenth day and the response are inversely proportional to the degree of anemia.[16] In our study there was slightly increase of reticulocyte count after supplementation but not significant and it was higher in iron plus placebo group.

In children with low 'normal' hemoglobin (Hb 11.0-11.4 gm/dl), 28 percent showed a therapeutic response to iron with an increase in hemoglobin concentration of 1.0 g per deciliter or more.[16] Our findings showed significant improvement of hemoglobin concentration in both of group and it was slightly higher at iron plus placebo group.

When the initial hemoglobin or the hematocrit concentration is below normal, the diagnosis of iron-responsive anemia should be considered. The presence of low or low normal MCV and/or MCH in conjunction with anemia increases the likelihood diagnosis of iron deficiency anemia,[14] so that anemia caused by infection, chronic inflammatory disease, thalassemia mayor, and lead poisoning could be excluded.[16] MCV and MCHC in our study were normal at the baseline with slightly increase after supplementation because ferritin concentration was still normal.

Although severe anemia is a common complication and cause of death in acute malaria,[16] our data suggest that asymptomatic malaria did not much influence hemoglobin concentration because at baseline hemoglobin concentration and serum ferritin was normal. Hemoglobin maintains relationship with serum ferritin that is typically observed during iron deficiency.

Zinc protoporphyrin (ZnPP) 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 of impaired iron utilization, zinc becomes an alternative metal substrate for ferrochelatase, leading to an increase in ZnPP formation.[17]

Study by Lind T *et al*[10] summarized that combined iron and zinc supplementation did not give optimal result. The combined supplement showed no effect on hemoglobin, whereas the single iron supplement had a clinically and statistically significant effect. In this study the effect of zinc was not significant to increase hemoglobin and reticulocyte. This might be caused by the normal baseline laboratory. The addition of zinc to iron supplementation did not affect heme biosynthesis and the binding between protophorphyrin and iron was not disturbed.

INACG (International Nutritional Anemia Consultative Group) in collaboration with WHO and UNICEF should review new information from analyses of existing data, result of trials in progress and early experience with program implementation. Oral iron supplementation program in malaria areas should be actively promoted.[18]

Iron is usually given as ferrous sulphate tablets, which are relatively low-cost and easy to transport and store. Liquid form of iron may be more appropriate

for children under two years because it is easier to administer and may give fewer adverse gastrointestinal effects.[12] In this study we also gave ferrous sulphate syrup for all children because it is easier to administer and more attractive for children and parents.

We suggest that iron supplementation for asymptomatic malaria with normal serum ferritin concentration is unnecessary as a prophylaxis for iron deficiency anemia caused by malaria.

In summary, combined iron and zinc supplementation in the treatment of children with malaria does not give optimal result. The combined supplement shows no effect in increasing reticulocyte and hemoglobin concentration in children with positive *Plasmodium falciparum.* Iron supplementation, with or without zinc, increases hemoglobin concentration significantly.

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