

Correlation between vivax malaria infection and iron deficiency in children

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Abstract

Background Iron deficiency is considered to be a major public health problem around the world due to its high prevalence as well as its effect on growth, development, and infection-resistance in children. In malaria-endemic areas, malaria infection is thought to contribute to the occurrence of iron deficiency, by means of hepcidin and hemolysis mechanisms.

Objective To assess the prevalence of asymptomatic vivax malaria, compare hemoglobin levels and iron status parameters between vivax malaria-infected and uninfected children, assess the prevalence of iron deficiency, and evaluate a possible correlation between vivax malaria infection and iron deficiency.

Methods This cross-sectional study was conducted from February to April 2013 at Sanana City of Sula Islands District, North Maluku. Six parameters were evaluated in 5-11-year-old children: malaria parasite infection, hemoglobin level, serum iron concentration, total iron-binding capacity (TIBC), serum transferrin saturation, and serum ferritin concentration.

Results Among 296 children aged 5-11 years, 75 (25.3%) were infected with *Plasmodium vivax*. In infected children, hemoglobin, serum iron, transferrin saturation, TIBC and serum ferritin were significantly lower than in non-infected children ($P < 0.01$). Using a serum ferritin cut-off of $< 15 \mu\text{g/dL}$, 142 (48.0%) of the children were found to be iron deficient. There was a strong correlation between vivax malaria infection and iron deficiency (OR 3.573; 95%CI 2.03-6.29).

Conclusion The prevalence of asymptomatic vivax malaria infection was 25.3%. The hemoglobin level and iron status parameters in vivax malaria-infected subjects were significantly lower than in uninfected children. The prevalence of iron deficiency was 48.0% for all study subjects. Malaria vivax infection was correlated with iron deficiency in 5-11-year-old children at Sanana City. [Paediatr Indones. 2015;55:44-9].

Keywords: *Vivax malaria, iron deficiency, malaria-endemic area, cross-sectional*

Iron deficiency is considered to be a major public health problem and the most common nutritional deficiency worldwide. Currently, 2 billion people are estimated to suffer from anemia, and approximately 90% of anemia cases are due to iron deficiency.¹ Iron deficiency is caused by a negative balance between requirements and bioavailability of iron.² Risk factors for iron deficiency include limited access to food, low-iron or other specialized diets, and medical conditions that affect iron status, such as parasitic infections.³ Malaria is also thought to contribute to the occurrence of iron deficiency through hepcidin and hemolysis mechanisms.⁴ Studies in African countries showed that malaria had a correlation with iron deficiency.^{5,6} The proportion of iron deficiency anemia in malaria-infected children was reported to be significantly higher than in uninfected children.⁷ In Indonesia, there has been little data about a correlation between malaria and iron deficiency in children.⁸ A study in West Sumba shows the hepcidin concentration and iron parameter status of subjects with malaria.⁹ Data

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from a pilot project study in children in the endemic area of Sanana City showed that hemoglobin level and serum iron concentration in malaria-infected children were lower than in uninfected children.¹⁰

We performed a cross-sectional design to assess for a correlation between vivax malaria infection and iron deficiency in children in the malaria-endemic area of Sanana City, Sula Islands District, North Maluku. We aimed to assess the prevalences of asymptomatic vivax malaria infection, iron depletion, iron deficiency, and iron deficiency anemia, as well as to compare hemoglobin level, serum iron concentration, TIBC, transferrin saturation, serum ferritin concentration and stage of iron deficiency in malaria-infected and uninfected children.

Methods

This study was conducted in Sanana City, Sula Islands District, North Maluku. The geographical location of Sula Islands District is latitude 01°4'00"-02°15'00" N and longitude 124°05'00"-126°50'00" E. Children were recruited from February to April 2013. There was no record of a previous survey of this nature conducted in this area and the prevalences of malaria and iron deficiency were unknown.

This study was cross-sectional and prospective in design. Children were randomly selected based on a multistage random sampling within the prescribed area. The elementary schools were visited and the purpose of the study made known to parents and students. Eligible study participants were aged 5 to 11 years and had parental consent. We excluded children with a history of prematurity or low birth weight, severe anemia, severe malnourishment, axillary temperature >37.5°C, infections of falciparum and ovale malaria, mixed malaria, or those who refused to participate.

A research laboratory in a clinic setting was established in the area and children were recruited from the elementary school within the study area. Children included in this study received free laboratory tests and medical care for malaria.

We collected 2 mL venous blood specimens in ethylene diamine tetracetic acid (EDTA) bottles for malaria and hematological investigations. Three mL of clotted blood was centrifuged and the serum used

for biochemical studies.

Hemoglobin level was determined using the cyanmethemoglobin method with an *Auto Hematology Analyzer MS 4-20*. Serum iron concentration and total iron-binding capacity (TIBC) were determined using a colorimetric method with Siemens *Dimension Xpand Plus® Integrated Chemistry System (Siemens DF85 no. 10444945 and DF84 no. 10444944)*. Serum transferrin saturation was calculated as a percentage of the total iron concentration in serum divided by the TIBC. The serum ferritin concentration was determined using an immunometric assay with *Siemens Dimension Xpand Plus® Integrated Chemistry System (Siemens RF440 no. 10444946)*. Test procedures were followed according to manufacturers' standard operating manual inserted in the kits.

Malaria infection was determined by microscopy with thick and thin smears using 100x objective oil immersion light microscopy. Thick and thin blood smears were stained with Giemsa stain according to standard procedures and examined by a certified laboratory analyst.

Asymptomatic vivax malaria was defined as infected by *Plasmodium vivax* with an axillary temperature <37.5°C. Iron deficiency was defined as serum ferritin <15 µg/dL. Iron depletion was defined as hemoglobin level ≥11.5 g/dL, serum ferritin <15 µg/dL and transferrin saturation 20-30%. Erythropoiesis iron deficiency (iron deficiency) was defined as hemoglobin level ≥11.5 g/dL, serum ferritin <15 µg/dL and transferrin saturation 10-20%. Functional iron deficiency (iron deficiency anemia) was defined as hemoglobin level <11.5 g/dL, serum ferritin <15 µg/dL and transferrin saturation <10%.

Subjects' parents provided written informed consent. The study was approved by the Ethics Committee of the Medical Faculty of Sriwijaya University and was approved by the National Unity, Political and Public Protection Agency of Sula Islands District.

Data were arranged in a 2x2 contingency table and analyzed using the *Statistical Package for Social Sciences (SPSS) version 16.0*. Hemoglobin and iron parameters were expressed as means and 95% confidence intervals. The T-test was used for parametric data and the Chi-square test was used for categorical data. A P value < 0.05 was considered as statistically significant.

Results

Of the 325 eligible children, 296 were analyzed and 29 were excluded (Figure 1). The 296 subjects comprised 132 boys and 164 girls, with a ratio of 1:1.2 boys to girls. The ratio of subjects aged 5-7 to those aged 8-11 years was 1:2.1. The majority (55.1%) of children in the study had good nutrition. Seventy-five children were infected with *Plasmodium vivax*, a prevalence rate of 25.5% (Table 1).

The mean hemoglobin level and iron parameters in infected and non-infected children are shown in Table 2. The hemoglobin level in infected children was significantly lower than in uninfected children ($P=0.001$). Similarly, serum iron concentration, TIBC, serum transferrin saturation and serum ferritin concentration in infected children were significantly lower than in uninfected children ($P<0.001$).

The prevalence of iron deficiency in the study was 48.0%, and the prevalence of iron deficiency in infected children was 70.7%. For our subjects, the proportions of iron depletion, iron deficiency and iron deficiency anemia were 9.5%, 25.7% and 12.8%, respectively (Table 3). The proportions of iron depletion and iron deficiency in infected children were not significantly higher than in uninfected children

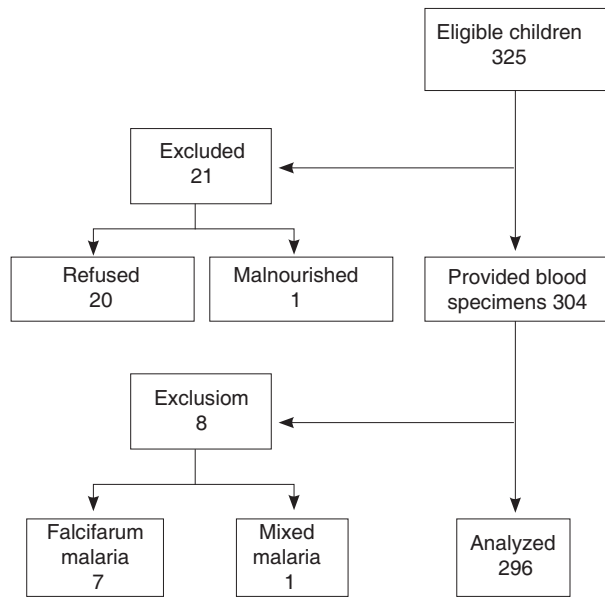


Figure 1. Flowchart of subject recruitment

($P>0.05$), however, the proportion of iron deficiency anemia in infected children was significantly higher than in uninfected children ($P<0.05$). In our study, vivax malaria had a significant correlation with iron deficiency (OR 3.57; 95%CI 2.03-6.29) (Table 4).

Table 1. Clinical characteristics of subjects (n=296)

	Infected (n=75)	Non-infected (n=221)	Total (n=296)
Gender			
Male, n (%)	35 (46.7)	97 (43.9)	132 (44.6)
Female, n (%)	40 (53.3)	124 (56.1)	164 (55.4)
Age group			
5-7 years, n (%)	26 (34.7)	70 (31.7)	96 (32.4)
8-11 years, n (%)	49 (65.3)	151 (68.3)	200 (67.6)
Nutritional status, n (%)			
Undernutrition	33 (44)	76 (34.4)	109 (36.8)
Good nutrition	41 (54.7)	122 (55.2)	163 (55.0)
Overweight	1 (1)	23 (10.4)	24 (8.1)

Table 2. Mean hemoglobin levels and iron parameters in infected and non-infected children (n=296)

Parameters	Infected children (n=75)	Uninfected children (n=221)	P value
Mean hemoglobin (range), g/dL	11.8 (11.6-12.0)	12.2 (12.1-12.3)	0.001
Mean serum iron (range), µg/dL	65.2 (57.1-73.2)	88.6 (84.6-92.6)	0.000
Mean total iron binding capacity (range), µg/dL	373.2 (356.7-389.7)	397.1 (389.0-405.3)	0.005
Mean transferrin saturation (range), %	17.6 (15.5-19.8)	22.7 (21.6-23.8)	0.000
Mean serum ferritin (range), µg/dL	20.1 (12.8-27.5)	34.8 (30.3-39.3)	0.001

Table 3. Iron deficiency stages in infected and uninfected children

	Infected children (n=75)	Uninfected children (n=221)	Total (n=296)
Iron deficiency			
Iron depletion, n (%)	8 (10.7)	20 (9.1)	28 (9.5)
Iron deficiency, n (%)	24 (32)	52 (23.5)	76 (25.7)
Iron deficiency anemia, n (%)	21 (28)	17 (7.7)	38 (12.8)
No iron deficiency, n (%)	22 (29.3)	132 (59.7)	154 (52.0)

Table 4. The correlation between vivax malaria infection and iron deficiency (n=296)

	Iron deficiency		Total	OR	95% CI
	Yes	No			
Infected	53	22	75	3.57	2.03-6.29
Uninfected	89	132	221		

Discussion

The prevalence of asymptomatic vivax malaria in children at Sanana City was 25.3%. This result was higher than the corresponding prevalence of 11.2% from *Ditjen PP & PL Kemenkes RI* blood evaluation of the mass population in North Maluku in 2008.¹¹ The proportion of vivax malaria infection were similar in both genders and age groups. However, the proportion of vivax malaria infection was higher in children with undernutrition. Malaria infection was reported to be impacted by immune status, parasite density, genus and strain of *Plasmodium*, nutritional status, and anti-malaria prophylaxis.¹²

The mean hemoglobin level in malaria-infected children was lower than in uninfected children, similar to a study by Jeremiah *et al.* in 1 to 8-year-old children in Nigeria.⁷ Anemia in malaria infection is complicated by premature destruction of malaria-infected and uninfected erythrocytes by macrophages, decreased production of erythrocytes from bone marrow suppression, and iron redistribution to macrophages.¹³ In vivax malaria infection, approximately 34 uninfected cells are cleared for every one infected cell destruction.¹⁴ Mean serum iron concentration, TIBC, serum transferrin saturation and serum ferritin concentration in malaria-infected children were significantly lower than in uninfected children, also similar to results from Jeremiah *et al.*⁷

In malaria, infected erythrocyte removal from circulation is followed by much uninfected red blood cell destruction. Both intra- and extra-vascular hemolysis occurs, increasing the clearance of plasma

heme by hemopexin and plasma hemoglobin by haptoglobin. Both haptoglobin-hemoglobin complexes and hemopexin-heme complexes prevent iron from hemoglobin breakdown to be recycled.¹⁵ The malaria parasite also ingests as much as 80% of hemoglobin into an acidic food vacuole, where the globin protein is digested and heme is released. More than 95% of the heme released from host hemoglobin by malaria parasites is detoxified by aggregation of the insoluble, chemically inert hemozoin in a crystallized form within lipid bodies. Hemozoin is resistant to degradation by heme oxygenase and accumulates in macrophages, monocytes, and polymorphonuclear leukocytes as non-bioavailable deposits that may persist for months.⁴

Serum ferritin <15 µg/dL in 5 to 11-year-old children was a specific predictor for iron deficiency. In this study, the prevalence of iron deficiency was 48.0%. A high prevalence of iron deficiency in 5 to 11-year-old children in Sanana City. Similar results were found by Onyemaobi *et al.* in children in an endemic area in Nigeria (48.8%)⁶ and in the 2007 Indonesian Basic Health Research report (47.2%).⁸ The prevalence of iron deficiency in infected children was even higher (70.7%), similar to the Onyemaobi *et al.* study (74.6%).⁶

Iron deficiency is caused by a negative balance between iron requirements and iron bioavailability.² Iron deficiency occurs in three stages.^{16,17,18} The first stage, iron depletion, occurs when iron content is not enough to meet body requirements, characterized by reduced iron deposition without functional changes, and serum ferritin <15 µg/L. The second stage,

iron deficiency, is characterized by a reduction in serum iron, serum transferrin saturation <16%, and an increase in free erythrocyte protoporphyrin level. In the third stage, iron deficiency anemia, the hemoglobin levels are below the standards for age and gender, and microcytosis and hypochromia develop.^{16,17} The proportions of iron depletion, iron deficiency and iron deficiency anemia in this study were 9.5%, 25.7% and 12.8%, respectively. The proportion of iron deficiency anemia in malaria-infected children was significantly higher than in uninfected children.

We observed a strong correlation between vivax malaria infection and iron deficiency in 5 to 11-year-old children. Onyemaobi *et al.* in Nigeria also found a strong correlation between malaria and iron deficiency in children under 5 years of age.⁶

In summary, we find that the prevalence of asymptomatic vivax malaria infection in 5 to 11-year-old children at Sanana City is 25.3%. The mean hemoglobin level and iron status parameters in malaria-infected children are lower than in uninfected children. The prevalence of iron deficiency in our subjects is 48.0%, and the proportions of iron depletion, iron deficiency and iron deficiency anemia are 9.5%, 25.7% and 12.8%, respectively. The proportion of iron deficiency anemia in malaria-infected children is higher than in uninfected children. Malaria vivax infection has a correlation with iron deficiency in children.

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