

## *Plasmodium falciparum* infection and the risk of anemia in school children

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### ABSTRACT

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#### BACKGROUND

According to the World Health Organization, malaria is the major cause of death from tropical infections in children and pregnant women. Severe anemia is a common complication of malaria, particularly malaria caused by *Plasmodium falciparum*. The purpose of the present study was to determine any association between *Plasmodium falciparum* infection and anemia in school children.

#### METHODS

This was an observational study with cross-sectional approach. A total of 540 whole blood samples of school children after administration of anthelmintic treatment for 30 days, were collected for measurement of hemoglobin concentration and preparation of Giemsa-stained thin and thick blood smears. The species of *Plasmodium* was confirmed by real-time polymerase chain reaction (PCR). Chi-square and prevalence ratio (PR) tests were used to determine the relationship between *Plasmodium falciparum* infection and anemia.

#### RESULTS

The prevalence of *Plasmodium* infection in school children of malarial endemic areas was 3.51%. There were 43 cases of anemia with 41 cases of mild anemia and 2 cases of moderate anemia. Among the 41 mild cases of anemia, *Plasmodium falciparum* infection was found in only 3 cases. The 2 cases of moderate anemia and the remaining 38 cases of mild anemia were not caused by *Plasmodium falciparum* infection. Subjects with *Plasmodium falciparum* infection had a 1.91-fold higher risk of suffering from anemia than subjects without *Plasmodium falciparum* infection, but not significant (PR=1.91; 95% C.I.=0.65-5.62).

#### CONCLUSIONS

*Plasmodium falciparum* infection did not significantly increase the risk of anemia in school children. Efforts to improve strategies for controlling anemia and malaria are needed.

**Key words:** *Plasmodium falciparum*, anemia, school children

## *Infeksi Plasmodium falciparum dan risiko terjadinya anemia pada anak sekolah*

### **ABSTRAK**

#### **LATAR BELAKANG**

World Health Organization menyatakan bahwa malaria merupakan penyebab kematian utama penyakit infeksi tropis pada anak-anak dan wanita hamil. Anemia berat merupakan komplikasi yang umum dari malaria, terutama malaria yang disebabkan oleh *Plasmodium falciparum*. Penelitian ini bertujuan untuk menentukan adanya hubungan antara infeksi *Plasmodium falciparum* dan anemia pada anak sekolah.

#### **METODE**

Penelitian ini merupakan penelitian observasional dengan pendekatan cross-sectional. Sejumlah 540 sampel darah anak sekolah yang telah mendapat terapi kecacingan selama 30 hari, diambil untuk pengukuran kadar hemoglobin dan pembuatan preparat malaria darah tebal serta darah tipis dengan pewarnaan Giemsa. Spesies *Plasmodium* dipastikan dengan menggunakan real time polymerase chain reaction (PCR). Digunakan uji chi-square dan rasio prevalensi (RP) untuk menentukan hubungan antara infeksi *Plasmodium falciparum* dengan anemia.

#### **HASIL**

Tingkat infeksi *Plasmodium* pada anak sekolah di daerah endemis malaria sebesar 3,51%. Terdapat 43 kasus anemia dengan kasus 41 anemia ringan dan 2 kasus anemia sedang. Dari total 41 kasus anemia ringan, infeksi *Plasmodium falciparum* hanya ditemukan pada 3 kasus. Dua kasus anemia sedang dan 38 kasus anemia ringan yang dialami oleh subyek ternyata bukan disebabkan oleh infeksi *Plasmodium falciparum*. Subyek yang terkena infeksi *Plasmodium falciparum* mempunyai resiko 4,22 kali untuk mengalami anemia dibandingkan dengan subyek yang tidak terinfeksi, tetapi tidak bermakna (RP=1,91; 95% C.I.=0,65-5,62).

#### **KESIMPULAN**

Infeksi *Plasmodium falciparum* tidak mampu secara bermakna meningkatkan risiko terjadinya anemia pada anak sekolah. Diperlukan upaya peningkatan strategi pengendalian anemia dan malaria.

**Kata kunci :** *Plasmodium falciparum, anemia, anak sekolah*

## **INTRODUCTION**

To date malaria is still the major infectious disease leading to high rates of disability and mortality in tropical and subtropical countries. The World Health Organization (WHO) recorded in 2008 an annual total of 247 million malaria cases worldwide, with one million deaths and 85% of these deaths occurring in the under-fives. This makes malaria a health problem in endemic countries that urgently needs attention and integrated management.<sup>(1)</sup>

A total of 3.3 milliard people throughout the world live in 450 malarial endemic countries.<sup>(1)</sup> The highest mortality rate, especially in the under-fives, occurs in the region of sub-Saharan Africa, while the second highest mortality rate is in Southeast Asia.<sup>(2)</sup> The manifestations of clinical malaria is variable, from asymptomatic parasitemia in semi-immune populations to severe malaria in susceptible populations, such as the under-fives, pregnant women, and nonimmune individuals. In susceptible populations, clinical malaria manifests itself in

various forms, such as cerebral malaria, severe anemia, metabolic acidosis, hypoglycemia, and other symptoms.<sup>(3,4)</sup> Cerebral malaria and severe anemia are the major causes of death in children. Cerebral malaria occurs more frequently in children older than 2 years in areas of low transmission. On the other hand, severe anemia commonly occurs in children under 2 years old in areas of high transmission.<sup>(5,6)</sup>

Severe anemia is a frequent complication of malaria, particularly malaria due to *Plasmodium falciparum*. The pathogenesis of anemia in malaria is at present unclear, since it is complex and multifactorial. Anemia results in a mortality rate of 15-20% among patients with malaria, in spite of their having received anti-malarial drugs. Damage to erythrocytes infected with *Plasmodium* is a minor factor contributing to the pathogenesis of malaria. It has been demonstrated that parasite density is not directly associated with the degree of anemia.<sup>(7,8)</sup> Presumably other factors contribute to the pathogenesis of malarial anemia, such as abnormal erythropoiesis in the bone marrow as a result of proinflammatory cytokines, excessive destruction of *Plasmodium*-infected or noninfected erythrocytes by splenic macrophages, and intravascular hemolysis of erythrocytes infected with the parasite.<sup>(9)</sup>

In sub-Saharan Africa more than 3.8 million children below the age of 10 years suffer from falciparum malaria and an estimated 2.6 million among these become anemic. Anemia in children with malaria is still a public health problem, since it affects their survival and development of cognitive functions. Low hemoglobin levels lead to impaired intellectual and motor functions of the children. Younger children will have a more severe anemia.<sup>(10)</sup>

The sub-district (*kecamatan*) of Nangapanda, Nusa Tenggara Timur, is an area endemic for malaria. The prevalence of *Plasmodium falciparum* and *Plasmodium vivax* is 12%. The total population of Kecamatan Nangapanda is 22,000 and 27% of

them are children of school-going age who are at risk for malarial infection.<sup>(11)</sup> With a population of school children constituting one-fourth of the total population of Kecamatan Nangapanda, it was considered necessary to conduct a study to determine any relationship between *Plasmodium falciparum* infection and anemia in school children.

## METHODS

### Design of the study

A study of observational cross-sectional design was conducted from June 2011 until January 2013.

### Study subjects

The subjects of this study were all of the 540 school children aged 5 to 18 years attending primary schools (SD), junior high schools (SMP) and senior high schools (SMU) in Kecamatan Nangapanda, Nusa Tenggara Timur.

### Measurement of hemoglobin concentration

Hemoglobin (Hb) concentration was measured with a Sysmex KX-21 hematology analyzer, using tubes containing 3 ml of blood with added EDTA as anticoagulant. Subjects were categorized as having anemia if the Hb concentration was <11g/dL.<sup>(12)</sup>

### Microscopic examination of blood

Detection of *Plasmodium* spp. in thin and thick blood smears was done conventionally by Giemsa staining.

### Real-time PCR

DNA was isolated from 200 µl whole blood using the QIAamp® DNA Mini Kit (QIAGEN, Jerman), according to the QIAGEN manual. Real-time PCR was performed with the HotstarTag mastermix kit (QIAGEN, Jerman). Specific primers for each gene were obtained from GenBank Accession. *P. falciparum* XS-probe was labelled with Yakima Yellow, and *P. vivax*

XS-probe was labelled with FAM. Amplification of the respective DNA samples was done by real-time PCR.

### Ethical clearance

This study obtained ethical clearance from the Faculty of Medicine, University of Indonesia. The Ende District Health Service was informed of this study and approved this study to be conducted at 21 schools in Kecamatan Nangapanda. All school children participating in this study returned the informed consent form signed by their parents or wards and received treatment against soil transmitted helminths.

### Statistical analysis

Chi-square and prevalence ratio (PR) tests were used to determine the relationship between *Plasmodium falciparum* infections and anemia, at a significance level of 0.05.

## RESULTS

There were in total 540 subjects, consisting of 417 male children and 123 female children. The age range of the subjects was from 5 to 18 years, with mean age of  $10 \pm 2.80$  years. The mean weight of the subjects was  $25.08 \pm 8.47$  kg, the lowest weight being 12 kg and the highest 62 kg.

Mean Hb concentration of the 540 study subjects was  $11.93 \pm 0.75$  g/dL. The lowest Hb concentration of all study subjects was 8.0 g/dl and the highest Hb concentration was 12.9 g/

dL. Mean Hb concentration of the male subjects was  $12.09 \pm 0.70$  g/dL, with their lowest Hb concentration being 8.8 g/dL and their highest 12.9 g/dL. Mean Hb concentration of the female subjects was  $11.37 \pm 0.64$  g/dL, while the lowest Hb concentration was 8.0 g/dL and the highest Hb concentration was 11.9 g/dL.

Among the 540 study subjects from whom blood samples had been collected for identification of *Plasmodium falciparum* by real-time PCR, 19 subjects (3.3%) were infected. There were 16 subjects (3.2%) with *Plasmodium falciparum* infection but without anemia, whereas 3 subjects (6.8%) had both *Plasmodium falciparum* infection and mild anemia. There was no significant association between *Plasmodium falciparum* infection and anemia category ( $p=0.4497$ ) (Table 1). The risk of anemia in subjects with *Plasmodium falciparum* infection was found to be 1.91-fold higher than the risk in subjects without anemia, but the increase was not significant ( $PR=1.91$ ; 95% Confidence Interval = 0.65-5.62) (Table 2).

## DISCUSSION

Our study results indicated that the risk of anemia in subjects with *Plasmodium falciparum* infection was 1.9.1 fold higher than the risk in subjects without anemia. Essentially similar results were obtained in a Kenyan study showing that infection with *Plasmodium* was the major cause of childhood anemia in the study area.<sup>(13)</sup>

Table 1. Association between *Plasmodium falciparum* infection and anemia category

Anemia	<i>Plasmodium falciparum</i> infection		p *
	Positive (n=19)	Negative (n=521)	
Non-anemia	16 (3.2%)	478 (96.8%)	0.4497
Mild anemia	3 (6.8%)	41 (93.2%)	
Severe anemia	0 (0.0%)	2 (100.0%)	

\*Chi-square test : Non significant

Non-anemia; Hb  $\geq 11.0$  g/dL; Moderate anemia: Hb : 9.5-10.9 g/dL; Severe anemia: Hb : 8.0-9.4 g/dL

Table 2. Association between *Plasmodium falciparum* infection and severe anemia

Pf infection*	Anemia status				Prevalence ratio (95% confidence interval)
	<11.0 g/dl		≥11 g/dl		
	n	%	n	%	
Pf(+)	3	15.8	16	84.52	1.91 (0.65 – 5.62)
Pf(-)	43	8.3	478	91.7	

\*Pf = *Plasmodium falciparum*

These mild *Plasmodium falciparum* infections were associated with anemia in school children. Halliday et al.<sup>(14)</sup> also found similar results indicating a strong association between *P. falciparum* and anemia in areas with moderate malarial transmission.

Malaria is a public health problem that may result in deaths, particularly in high risk groups, such as infants, the under-fives, and pregnant women, in addition to being a direct cause of anemia and capable of decreasing work productivity. The disease is still endemic in most areas of the Indonesian archipelago.<sup>(15,16)</sup> The focus of the present study was to determine the contribution of *Plasmodium falciparum* infection to anemia in school children in malarial endemic areas. To eliminate possible bias from soil-transmitted helminths, especially mine worms, as a cause of iron-deficiency anemia, with clinical manifestations similar to anemia from malaria, all school children received anthelmintic treatment before inclusion in this study. More than half of the deaths from malaria, particularly in children and pregnant women, are the result of severe anemia. Of all pathophysiological processes underlying severe malaria, the mechanism of anemia as one of the clinical signs of malaria is still not clearly understood. There have been many studies and hypotheses to explain anemia in malaria, but none of the suggested pathophysiological mechanisms of severe anemia are satisfactory.<sup>(8)</sup>

The WHO defines severe anemia in malaria as anemia with a hemoglobin concentration of <5 g/dL or a hematocrit of <15%.<sup>(12)</sup> Anemia in malaria leads to poor

oxygenation at tissue or cellular levels, due to poor oxygen uptake by the mitochondria and the resulting dysfunction of the whole cell.

In our study, mild and moderate anemia were almost uniformly distributed over the whole age range of the school children. This is in contrast with the results of a study conducted by Ngui et al. in West Malaysia Barat, where anemia was more common in preschool children. This was due to a low prevalence of anemia among school children in the study area, resulting from state-supported school nutrition programs.<sup>(17)</sup>

The program for enhancement of community nutritional status and the nutritional supplementation program carried out by the Ende District Health Service may presumably not yet have the school children in Kecamatan Nangapanda under coverage. There are to our knowledge no published studies on the occurrence of anemia among preschool children in Kecamatan Nangapanda.

The majority of the subjects with positive results for *Plasmodium falciparum* had no anemia. This may be firstly due to mild infections which do not result in anemia, and secondly, there is the possibility that the majority of subjects are adapted to malaria in the study area, which is endemic for malaria. Assuming that the possibility of the anemia being caused by mine worm infection has been eliminated, the factors presumably playing a role in cases of moderate anemia are nutrition and undiagnosed infections. The low socioeconomic status of Kecamatan Nangapanda may predispose to poor nutritional intake.<sup>(11)</sup> The weights of all school

children involved as study subjects are normal according to the z-score (data not shown), therefore nutritional factors presumably play a minor role in anemia incidence among these children. However, there are no reports of studies on the relationship of nutrition to anemia in school children in Kecamatan Nangapanda.

Other possible causes of anemia in general are other infections, such as pyogenic infections by gram-positive or gram-negative bacteria, mycobacterial infections, especially by *Mycobacterium tuberculosis*, brucellosis or even non-infectious factors such as arthritis.<sup>(18)</sup>

Although Indonesia is the third country in the world with a high prevalence of tuberculosis,<sup>(19)</sup> anemia in the school children of Kecamatan Nangapanda cannot with certainty be assumed to originate from infection with *Mycobacterium tuberculosis*, since all study subjects have a good nutritional status according to their z-score. Among the school children of Kecamatan Nangapanda, the causes of mild and moderate anemia cases that are not the result of *Plasmodium* infection, are as yet unknown and need to be further investigated.

All study subjects are original inhabitants of the district, with a high probability of harboring malarial infection. However, few subjects were found who were positive for *Plasmodium falciparum*, either by microscopy or real-time PCR. Using microscopy or real-time PCR on the 540 school children as study subjects, the infection rate of *Plasmodium falciparum* was found to be very low, even in areas endemic for malaria. The present study did not collect data on nutrition of the study subjects and data on other infections besides those by *Plasmodium*, thus the other possible causative factors of anemia could not be evaluated.

## CONCLUSIONS

*Plasmodium falciparum* infection did not increase the risk of anemia in school children. Efforts to improve strategies for controlling anemia and malaria are needed.

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