

Hemolytic anemia in falciparum and vivax malarial patients based on serum bilirubin examination

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ABSTRACT

Objective To examine hemolysis in falciparum and vivax malarial patients based on serum bilirubin examination.

Methods A cross sectional study was conducted on children younger than 15 years of age who visited public health center in the district of Mandailing Natal with complaints of fever, shivering, pale, jaundice, diarrhea, or headache between April 9th and April 19th 2001. Variables recorded were age, gender, body weight, body height, symptoms and signs, anti malarial drugs, and laboratory test results. Thin and thick blood smears were done as diagnostic tools of malaria. Thin blood smear was also performed to determine the level of malaria parasites in blood (parasitemia) and to examine the morphology of red blood cells. Hemolysis was determined by bilirubin examination.

Results In *P. falciparum* malaria, there was a moderate correlation ($r=0.68$, $p<0.0001$) between parasitemia and indirect bilirubin concentration. While in *P. vivax* malaria, there was only a weak correlation ($r=0.46$, $p=0.007$) between parasitemia and indirect bilirubin concentration. It was also found that in falciparum malaria, parasitemia, total and indirect bilirubin concentrations were significantly higher than that in vivax malaria, with p values of 0.009, 0.015 and 0.003, respectively.

Conclusion Hemolysis in falciparum malaria is more severe than that in *P. vivax* malaria, with marked elevation of indirect bilirubin. The elevation of serum bilirubin correlated with parasitemia [Paediatr Indones 2004;44:95-100].

Keywords: Hemolytic anemia, falciparum malaria, vivax malaria, serum bilirubin.

Malaria is still a major public health problem in Indonesia, especially out of Java and Bali.^{1,2} Hemolytic anemia is one of the most important complications, especially in children who live in endemic malarial area.^{3,4} Morbidity and mortality that

caused by anemia in malaria infection are very high.³ District of Mandailing Natal is one of the most endemic areas of malaria in North Sumatera province.⁵ Sporozoa of the genus Plasmodium as the etiologic agents of human malaria are transmitted to humans by female mosquitoes of the genus Anopheles.¹ Plasmodium falciparum and Plasmodium vivax are the predominant species in Indonesia including the district of Mandailing Natal.⁵

Hemolytic anemia is associated with high serum bilirubin level, especially serum indirect bilirubin^{1,6} The purpose of this study was to examine the hemolysis in falciparum and vivax malarial patients based on serum bilirubin examination.

Methods

A cross sectional study was conducted on children younger than 15 years of age, who visited Public health Center in the district of Mandailing Natal, North

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Sumatera province with complaints of fever, chills, pale, jaundice, diarrhea, or headache during the period of April 9th until April 19th 2001. Eligible patients were all falciparum and vivax malarial patients who did not get anti malarial drugs 1 week before study. Patients with hemolytic anemia due to any drugs, hepatitis, or hepatic carcinoma were excluded. Written informed consents were obtained from parents and guardians before enrollment. By using the formula,⁷ sample size calculated were 81 (95% confidence interval, 1.960 normal standard deviate of a, 0.842 normal standard deviate of b, 0.6 standard proportion, 0.8 proportion of *P. falciparum* or *P. vivax* patients, and 0.8 power). Subjects were selected by means of simple random sampling method.

The following variables were recorded: age, gender, body weight, body height, symptoms and signs, anti malarial drugs used and laboratory test results. Thin and thick blood smears were done as diagnostic tools of malaria. Thin blood smear was also performed to determine level of malarial parasites in blood (parasitemia) and to examine the morphology of red blood cells. Hemolysis was determined by using bilirubin examination. Bilirubin examinations including total bilirubin, direct and indirect bilirubin was carried out by using spectrophotometer and was performed twice (on D₀= at the time of diagnosis and on D₅= day 5 after got anti malarial drug). A cyanomethemoglobin method was used to determine hemoglobin level, whereas spectrophotometer was used to measure aspartate aminotransferase (AST) and alanine aminotransferase

(ALT). There were two kinds of anti malarial drugs used including chloroquine and Fansidar.

Data were recorded and analyzed by computer (SPSS version 10.0). Statistical comparison between quantitative variables was calculated using independent t-test. Correlation between hemolysis and parasitemia was analyzed by linear regression. A p value of <0.05 was considered to be statistically significant.

Results

Of one hundred and fifty two children, there were 49 children suffered from falciparum malaria and 18 children who suffered from vivax malaria. The characteristics of patients and antimalarial drugs used in the study are depicted in **Table 1**. The distributions of ages for all groups were relatively inequitable. One to five years old was the most frequent age in falciparum malaria, followed by the age of 5-10 years with the youngest patient were 6 month old. In malaria vivax, the greatest proportion of age was 5-10 years old, followed by the age of 1-5 years with the youngest patient of 9 month old. *Plasmodium falciparum* was the most frequent species found than *Plasmodium vivax*.

Clinical symptoms of falciparum malaria and vivax malaria were fever, followed by pale, headache and diarrhea. There were no significant differences between falciparum and vivax malarial patients from the point of view of fever, pale, diarrhea and headache ($p>0.05$). The most frequent physical findings

TABLE 1. CHARACTERISTIC OF PATIENTS AND KINDS OF ANTI-MALARIAL DRUGS

	Falciparum malaria (n = 49)	Vivax malaria (n = 18)
Gender		
- Female	20	10
- Male	29	8
Age (years)		
- ≤ 1	7	3
- 1 – 5	19	5
- 5 – 10	16	8
- 10 – 15	7	2
Body weight (kg)*	14.84 (SD 6.56)	16.22 (SD 9.03)
Body height (cm)*	96.51 (SD 24.91)	99.17 (SD 25.86)
Kinds of anti malarial drug		
- Chloroquine	17	18
- Fansidar	32	0

* Mean (standard deviation)

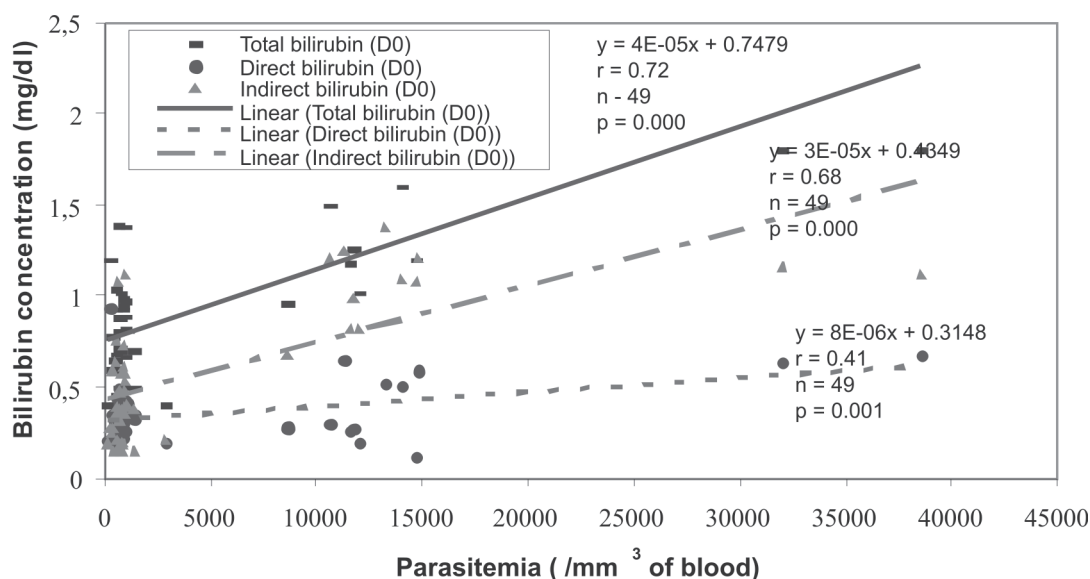


Figure 1. Scatter diagram of parasitemia to bilirubin concentrations in falciparum malarial patients on D₀

were fever, pale, splenomegaly, and hepatomegaly in either falciparum or vivax malarial patients.

Figure 1 shows scatter diagram of parasitemia to bilirubin concentration in falciparum malarial patients on D₀. An equation of serum total bilirubin, $y=4E-5x+0.7479$ ($n=49$), revealed strong correlation coefficient ($r=0.72$) between parasitemia and serum total bilirubin. An equation of serum indirect bilirubin, $y=3E-05x + 0.4349$ ($n = 49$), revealed strong correlation coefficient ($r = 0.68$) between parasitemia and serum indirect bilirubin, whereas, an equation of serum direct bilirubin, $y=8E-06x+0.3148$ ($n=49$), revealed sufficient correlation coefficient ($r=0.41$) between parasitemia and serum direct bilirubin. There were statistically significant differences with p values of <0.0001 , <0.0001 and 0.001 respectively.

Figure 2 also shows scatter diagram of parasitemia to bilirubin concentration in vivax malarial patients on D₀. An equation of serum total bilirubin, $y=4E-05x+0.649$ ($n=18$), revealed sufficient correlation coefficient ($r=0.48$) between parasitemia and serum total bilirubin. An equation of serum indirect bilirubin, $y=3E-05x+0.3657$ ($n=18$), revealed sufficient correlation coefficient ($r=0.46$) between parasitemia and serum indirect bilirubin, whereas an equation of serum direct bilirubin, $y=1E-05x+0.2894$ ($n=18$), revealed sufficient correlation coefficient between parasitemia and serum direct bilirubin. There

were statistically significant differences with p values of 0.006, 0.007 and 0.011 respectively.

Table 2 shows the laboratory findings in falciparum and vivax malarial patients. There were significant differences between falciparum and vivax malarial groups concerning parasitemia, total bilirubin and indirect bilirubin concentrations on D₀, whereas on D₅ there were not. However, concerning direct bilirubin concentration, there was no significant difference between both groups on D₀, but on D₅ there was a significant difference. The mean hemoglobin concentration, AST, ALT and platelet counts in both groups were still normal. The morphology of red blood cells in falciparum and vivax malarial patients was consistent with a hemolytic anemia.

Discussion

In this study, the distributions of ages for all groups were relatively inequitable. In *P. falciparum* malaria, 1-5 years old was the most frequent age, followed by the age of 5-10 years with the youngest patient of 6 month old. In vivax malaria, the greatest proportion of age was 5-10 years old, followed by the ages of 1-5 years with the youngest patient of 9 months old. It was not different with the study of Albar *et al* that

TABLE 2. LABORATORY FINDINGS IN FALCIPARUM AND VIVAX MALARIAL PATIENTS

Laboratory findings	Falciparum malaria Mean (SD)	Vivax malaria Mean (SD)	p
Parasitemia (/mm ³ of blood)			
- D ₀	4542 (SD 7997)	2374 (SD 3085)	0.009
- D ₅	81 (SD 232)	96 (SD 358)	0.518
Hb (g/dl)	10.46 (SD 1.48)	10.41 (SD 1.96)	0.505
Total bilirubin (mg/dl)			
- D ₀	0.93 (SD 0.43)	0.75 (SD 0.26)	0.015
- D ₅	0.57 (SD 0.23)	0.51 (SD 0.17)	0.277
Direct bilirubin (mg/dl)			
- D ₀	0.35 (SD 0.15)	0.31 (SD 0.10)	0.140
- D ₅	0.28 (SD 0.14)	0.24 (SD 0.06)	0.007
Indirect bilirubin (mg/dl)			
- D ₀	0.58 (SD 0.36)	0.43 (SD 0.20)	0.003
- D ₅	0.29 (SD 0.17)	0.27 (SD 0.17)	0.657
AST (U/L)	45.02 (SD 18.73)	39.11 (SD 12.99)	0.715
ALT (U/L)	48.53 (SD 20.23)	42.89 (SD 13.54)	0.487
Thrombocyte (/mm ³)	221735 (SD 58361)	229667 (SD 43155)	0.109
Morphology of blood cells	49	18	
Hemolytic anemia (n)			

found 57.89% patients with an age of <5 years old and the youngest patient of 5 months of age.⁸

The results of thin and thick blood smear examinations revealed *P. falciparum* (49 patients) as the most frequent species, while *P. vivax* were found in 18 patients, and there were no other *Plasmodium* noted. Oemijati¹ and Rampengan² found that *P. falciparum* and *P. vivax* were the predominant species in Indonesia. A malariometric survey done in 11 districts in Sumatera Utara province since 1990-1993 found 2 species i.e., *P. falciparum* and *P. vivax*.⁵

According to our observation, most clinical symptoms in *P. falciparum* and *P. vivax* malarial patients were fever (95.9% vs 94.4%), pale (71.4% vs 66.6%), headache (46.9% vs 50.0%), and diarrhea (40.8% vs 50.0%). A study by Albar *et al*⁸ showed that the frequent clinical symptoms were fever (100%), vomiting (55.25%), shivering (42.11%), cough (34.21%) and diarrhea (31.58%).

Thirty-eight (77.5%) *P. falciparum* malarial patients and thirteen (72.2%) *P. vivax* malarial patients showed pale. Pale was the second frequent physical findings after fever (>37.5°C). This result was not different with the study of Albar *et al* that showed 89.47% anemia in malarial patients.⁸

The degree of anemia correlates with parasitemia and schizontemia. In *P. falciparum* malaria, anemia can develop rapidly due to profound hemolysis.⁶ Hemolysis is also associated with high serum bilirubin. The

increased serum bilirubin in hemolysis almost always consists of the unconjugated (indirect) pigment. The conjugated (direct) fraction remains within normal limits.^{1,4} In this study, it was also found that serum total and serum indirect bilirubin in *P. falciparum* malaria increased linearly with the increased parasitemia, which had strong correlation (Figure 1). Correlation coefficient between serum total or serum indirect bilirubin and parasitemia in *P. vivax* malaria showed only moderate correlation (Figure 2). This finding was an evidence that hemolysis in *P. falciparum* malaria was significantly more severe than that in vivax malaria.

Plasmodium falciparum infects immature and mature erythrocytes¹ and its parasitemia can be 20-30%.⁶ Whereas *Plasmodium vivax* mostly infects immature erythrocytes¹ and usually results in parasitemias of <2%.⁶ In our study, parasitemia in *P. falciparum* malaria was higher than that in *P. vivax* malaria and the difference of both groups was significant on D₀ (p=0.009). Five days after the patients had got anti malarial drugs (chloroquine or Fansidar), parasitemia and serum bilirubin concentrations (total and indirect bilirubins) in both groups reduced and returned to normal. Kakkilaya⁵ reported that in most malarial patients, serum bilirubin level usually returns to normal within 3-5 days of effective anti malarial treatment.

In this study, we did not find jaundice either in *P. falciparum* malaria or *P. vivax* malaria patients.

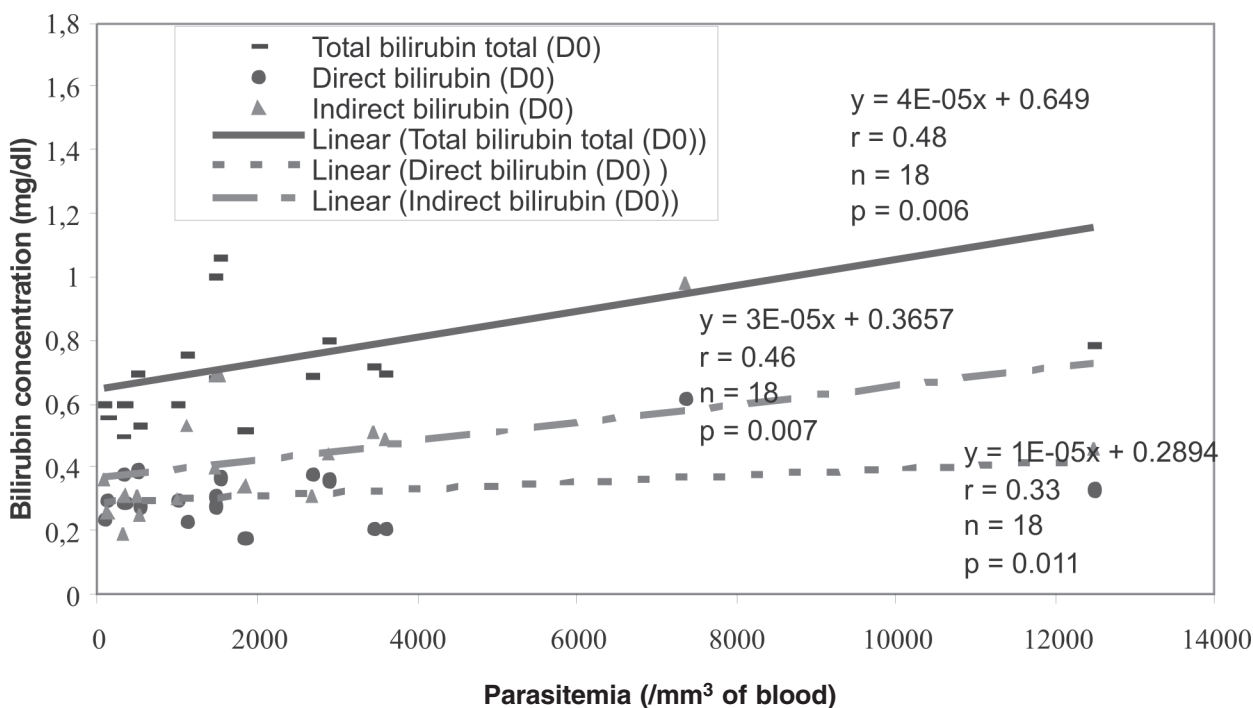


Figure 2. Scatter diagram of parasitemia to bilirubin concentrations in vivax malarial patients on D₀

Alwi Datau *et al*⁹ found hemolytic jaundice in about 17.2% of cases. According to the literature mild jaundice is fairly common in malaria and may be seen in 20-40% of the cases.^{6,10} Jaundice is seen when serum total bilirubin concentration rises 2-3 mg/dL.¹¹ Marked jaundice with serum total bilirubin of more than 3 mg/dl is seen in severe *P. falciparum* malaria.⁶

Hepatic dysfunction may also be seen in cases of severe *P. falciparum* malaria. Such patients have conjugated hyperbilirubinemia, marked elevation of AST and ALT.⁶ AST and ALT examinations which were performed on D₀ revealed normal results in all children either in *P. falciparum* or *P. vivax* malaria. Conjugated (direct) bilirubin concentration was also still normal in both groups either on D₀ or D₅. It showed that liver functions of all patients were normal. Alwi Datau *et al*⁹ found that the mean elevation of AST was 121 mU/ml and ALT 80.8 mU/ml.

We concluded that hemolysis in *P. falciparum* malaria was more severe than that in *P. vivax* malaria with marked elevation of indirect bilirubin. The elevation of

serum bilirubin correlated well with parasitemia. Liver functions of all patients were still normal.

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