

Coexistence of Malaria and Thalassemia in Malaria Endemic Areas of Thailand

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Abstract: Hemoglobinopathy and malaria are commonly found worldwide particularly in malaria endemic areas. Thalassemia, the alteration of globin chain synthesis, has been reported to confer resistance against malaria. The prevalence of thalassemia was investigated in 101 malaria patients with *Plasmodium falciparum* and *Plasmodium vivax* along the Thai-Myanmar border to examine protective effect of thalassemia against severe malaria. Hemoglobin typing was performed using low pressure liquid chromatography (LPLC) and α -thalassemia was confirmed by multiplex PCR. Five types of thalassemia were observed in malaria patients. The 2 major types of thalassemia were Hb E (18.8%) and α -thalassemia-2 (11.9%). There was no association between thalassemia hemoglobinopathy and malaria parasitemia, an indicator of malaria disease severity. Thalassemia had no significant association with *P. vivax* infection, but the parasitemia in patients with coexistence of *P. vivax* and thalassemia was about 2-3 times lower than those with coexistence of *P. falciparum* and thalassemia and malaria without thalassemia. Furthermore, the parasitemia of *P. vivax* in patients with coexistence of Hb E showed lower value than coexistence with other types of thalassemia and malaria without coexistence. Parasitemia, hemoglobin, and hematocrit values in patients with coexistence of thalassemia other than Hb E were significantly lower than those without coexistence of thalassemia. Furthermore, parasitemia with coexistence of Hb E were 2 times lower than those with coexistence of thalassemia other than Hb E. In conclusion, the results may, at least in part, support the protective effect of thalassemia on the development of hyperparasitemia and severe anemia in malaria patients.

Key words: *Plasmodium falciparum*, *Plasmodium vivax*, malaria, thalassemia, hemoglobin E

INTRODUCTION

Malaria is an ancient disease that causes death until present age. Resistance of malaria parasite to most of the available antimalarial drugs has been a major public health concern particularly in Southeast Asia. *Plasmodium falciparum* and *Plasmodium vivax* are the 2 predominant malarial species in this area [1]. Several host factors have been reported to contribute to malaria severity. These include innate immunity [2], hemoglobinopathies such as thalassemia [3], enzymopathies such as glucose-6-phosphate dehydrogenase (G6PD) deficiency [4], heme oxygenase (HO) polymorphism [5,6], and tumor necrosis factor- α promoter polymorphism (TNF- α) [7]. Among them, thalassemia has been reported to confer protection

against malaria disease [3].

Thalassemia is a hemoglobin disorder caused by alteration in the synthesis of globin chain of hemoglobin. This hemoglobinopathy is classified into 2 forms according to the abnormality of globin chains, i.e., α - and β -thalassemia. The α -thalassemia is a result of decrease in synthesis of 1 or 2 α -globin chain on chromosome 16p13.3 [8]. The deletion of α -globin gene results in alteration of the normal genotype ($\alpha\alpha/\alpha\alpha$) to the severe form, α -thalassemia-1 ($--/\alpha\alpha$, $--/--$), and the mild form, α -thalassemia-2 ($-\alpha/\alpha\alpha$, $-\alpha/-\alpha$). Furthermore, α -globin chain is also inactivated by point mutation. Similarly to α -thalassemia, β -thalassemia is the disorder produced by decrease in synthesis of β -globin chain which located on chromosome 11 (11p15.5) [8]. The common abnormality of β -globin gene is usually resulted from mutation rather than deletion. Hemoglobin E (Hb E), the most common form of thalassemia in Southeast Asia [9], is caused by replacement of glutamic acid at codon 26 of β -globin chain with lysine [10].

Thalassemia is widely distributed around the world and is commonly observed in malaria-endemic areas [11]. The coex-

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istence of thalassemia and malaria was reported to protect the infected host against malaria caused by *P. falciparum* [8,12-14]. Such association, however, was not demonstrated in *P. vivax* in a study from Papua New Guinea in children aged 3-21 months, although parasite density was significantly higher in α -thalassemia-2 [15]. To investigate the protective effect of thalassemia against malaria, the prevalence of thalassemia was investigated in patients infected with the 2 predominant malaria species, *P. falciparum* and *P. vivax*, in malaria endemic areas on the western and southern regions of Thailand.

MATERIALS AND METHODS

Study subjects and sample collection

A total of 101 blood samples were collected from patients (12 Thais and 89 Burmeses) with mono-infection with *P. falciparum* (n=40), *P. vivax* (n=58), or mixed infection of *P. falciparum* and *P. vivax* (n=3). The study was conducted at malaria clinics in Tak (Mae Sot district: n=69) and Ranong (Kraburi district: n=32) provinces of Thailand during 2012 and 2013. The study protocol was approved by the Ethics Committee of Thammasat University (Certificate no. 040/2555). Giemsa-stained thin and thick blood smears were prepared and examined microscopically for the presence of malaria parasites. Parasites and white blood cells (WBCs) were counted on the thick film using a 100 \times oil-immersion objective, and the number of parasites was recorded when 200 WBCs were counted.

Detection of thalassemia

Hemoglobin analysis was screened using osmotic fragility test (OF test), and hemoglobin typing was performed using automated low pressure liquid chromatography (LPLC) (auto-

mated analyzer: Hb Gold, Drew Scientific Ltd., Cumbria, UK). The genomic DNA was extracted from peripheral blood using a QIAamp DNA extraction mini-kit (QIAGEN, Valencia, California, USA) according to the standard protocol. The common α -thalassemia including α -thalassemia-1 (SEA and THAI deletions), α -thalassemia-2 (3.7 and 4.2 kb deletions), Hb constant spring, and Hb Pakse mutations were analyzed by multiplex PCR methods.

Statistical analysis

Statistical analysis was performed using the SPSS statistical package (version 12.0 SPSS Inc., Chicago, Illinois, USA). Difference of qualitative and quantitative data between groups was analyzed using the chi-square test and ANOVA with Tukey analysis, respectively. Statistical significance level was set at $\alpha=0.05$.

RESULTS

The prevalence of thalassemia in malaria patients

The typing of thalassemia was performed by determination of amount and type of Hb with Hb chromatogram, OF test, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). Normal or non-clinically significant thalassemia was identified using negative OF test, HbA₂ (<4%), MCV (≥ 80 fL), and MCH (≥ 27 pg). α -thalassemia-1 or 2 trait and homozygous α -thalassemia-2 were identified using positive OF test, HbA₂ (<4%), MCV (≥ 80 fL), and MCH (≥ 27 pg). β -thalassemia trait was identified using the criteria similarly to that of α -thalassemia-1 or 2 trait, except that the cut-off HbA₂ used was 4-8%. Hb E trait was identified using negative or positive OF test, Hb E ($\leq 25\%$), MCV (<80 fL or normal),

Table 1. Prevalence of thalassemia in patients with malaria in the western (Tak province) and southern (Ranong province) regions of Thailand

	Western region				Southern region				Summation (%)
	<i>P. falciparum</i>	<i>P. vivax</i>	Mixed	Total	<i>P. falciparum</i>	<i>P. vivax</i>	Mixed	Total	
Malaria with thalassemia									
Hb E ^a	3	9	2	14	5	0	0	5	19 (18.8)
β -thalassemia	1	0	0	1	0	0	0	0	1 (1.0)
α -thalassemia 2 ^a	3	6	0	9	2	1	0	3	12 (11.9)
Hb E/ α -thalassemia 2	0	1	0	1	0	0	0	0	1 (1.0)
β -thal/ α -thalassemia 2	1	2	0	3	1	0	0	1	4 (4.0)
Malaria alone	18	23	0	41	6	16	1	23	64 (63.4)
Total	26	41	2	69	14	17	1	32	101 (100)

Data are presented as numbers (%).

^aIncluding individuals with heterozygous and homozygous.

and MCH (<27 pg or normal); if the Hb E was less than 25%, it can be classified as Hb E trait with or without α -thalassemia. Homozygous Hb E with or without α -thalassemia was identified using positive OF test, Hb E ($\geq 80\%$), Hb F ($\leq 5\%$), MCV (<80 fL), and MCH (<27 pg).

The prevalence of thalassemia in 101 patients with malaria infection was 36.7%. Five types of thalassemia were observed, i.e., Hb E, β -thalassemia, α -thalassemia-2, Hb E/ α -thalassemia-2, and β -thalassemia/ α -thalassemia-2 (Table 1). The 2 major types were Hb E (18.8%) and α -thalassemia-2 (11.9%). The α -thalassemia-2 of all patients was a 3.7 kb deletion. The α -thalassemia-1 was not detected in any patient. The prevalence of Hb E vs α -thalassemia-2 thalassemia in Thai and Burmese patients were 2/12 (16.7%) vs 2/12 (16.7%) and 17/89 (19.1%) vs 15/89 (16.9%), respectively.

Relationship between malaria patients with and without coexistence of thalassemia

The malaria parasitemia in patients with and without coexistence of all 5 types of thalassemia were comparable (Table 2). No significant difference in parasitemia, hemoglobin, and hematocrit was observed between patients with malaria (both *P. falciparum* and *P. vivax*) with or without coexistence of thalassemia. However, the coexistence patients had 2 times lower parasitemia than malaria patients without coexistence. The prevalence of patients with and without coexistence of thalassemia in 2 endemic areas were 28 (40.6%) and 41 (59.1%) in western region and 9 (28.1%) and 23 (71.9%), respectively.

The *P. falciparum*-infected patients with coexistence of thalassemia showed 3 times lower parasitemia than *P. falciparum*-

infected patients without coexistence (Table 2). High parasitemia was found in patient with coexistence of α -thalassemia-2. Whereas, *P. vivax*-infected patients with and without coexistence of thalassemia showed similar parasitemia. Furthermore, mixed infection of *P. falciparum* and *P. vivax* patients with coexistence of thalassemia represented closely 3 times lower parasitemia than mixed infection patients without coexistence. The results might suggest the protective effect of thalassemia on *P. falciparum*-infected patients but not on *P. vivax*-infected patients. Nevertheless, there was no significant difference in parasitemia, hemoglobin, and hematocrit between malaria (both *P. falciparum* and *P. vivax*) patients with or without coexistence of thalassemia.

The comparison of *P. falciparum* with coexistence of thalassemia and *P. vivax* with coexistence of thalassemia showed 3 times lower parasitemia in *P. vivax* patients. The coexistence of β -thalassemia was found only in *P. falciparum* infection whereas Hb E/ α -thalassemia-2 was found only in *P. vivax* infection.

Relationship between Hb E thalassemia and malaria parasitemia, hemoglobin, and hematocrit

The malaria patients with coexistence of Hb E showed more prevalence than other types of thalassemia; therefore, parasitemia, hemoglobin, and hematocrit were compared. Parasitemia of malaria in patients with coexistence of Hb E showed lower than those with coexistence with other types of thalassemia and malaria without coexistence, 2 and 3 times, respectively (Table 3). Parasitemia, hemoglobin, and hematocrit values in patients with coexistence of thalassemia other than Hb

Table 2. Malaria parasitemia in patients with or without thalassemia

	Number of patients	Parasitemia (parasite/ μ l)			
		Total	<i>P. falciparum</i>	<i>P. vivax</i>	Mixed infection
Coexistence of malaria with thalassemia	37	15,981 (7,247-81,661)	24,697 (2,500-151,086)	8,525 (280-42,988)	17,080 (11,439-22,720)
Hb E	19		17,563 (4,444-72,158)	4,888 (865-12,075)	17,080 (11,439-22,720)
β -thalassemia	1		2,500	-	-
α -thalassemia 2	12		42,545 (9,703-151,086)	12,256 (280-42,988)	-
Hb E/ α -thalassemia 2	1		-	14,720 (14,720)	-
β / α -thalassemia 2	4		19,711 (17,631-21,790)	8,738 (4,667-12,808)	-
Malaria alone	64	37,207 (7,200-24,761)	83,496 (355-1,427,200)	8,386 (50-74,667)	50,320

Data are presented as mean (95% CI) values.

Table 3. Malaria parasitemia, hemoglobin, and hematocrit in patients with hemoglobin E thalassemia, other types of thalassemia, and those without thalassemia

	Number of patients	Parasitemia (parasite/ μ l)	Hb (g/dl)	Hct (%)
Coexistence of malaria with Hb E	19	11,508 (4,227- 27,243)	11.63 (9.69-13.56)	35.53 (29.50-41.56)
Coexistence of malaria with other types of thalassemia	18	20,702 ^a (13,366-54,770)	10.52 ^b (7.72-13.32)	32.39 ^c (24.17-40.61)
Malaria alone	64	37,207 (14,0758-215,173)	12.25 (9.87-14.63)	37.28 (30.12-44.44)

Data are presented as mean (95% CI) values.

Hb, hemoglobin; Hct, hematocrit.

^aStatistically significant difference with patients without thalassemia ($P=0.048$)

^bStatistically significant difference with patients without thalassemia ($P=0.021$)

^cStatistically significant difference with patients without thalassemia ($P=0.018$)

E were significantly lower than those without coexistence of thalassemia (parasitemia: $P=0.048$; hemoglobin: $P=0.021$; hematocrit: $P=0.018$). There was no significant relationship for such values in malaria patients with Hb E thalassemia.

DISCUSSION

Tak province in the western region and Ranong province in the southern region are the 2 top 10 provinces of Thailand with malaria incidence in 2013. Most of the patients included in the study were Burmese, and the prevalence rate of *P. vivax* infection was found to be higher than *P. falciparum* infection with the ratio 1.5:1. The overall prevalence rate of thalassemia hemoglobinopathy in this group of populations was 36.7%. The most prevalent type Hb E (18.8%) is a common abnormal hemoglobin form of thalassemia which is distributed in Mediterranean, East Asia, India subcontinent, and Southeast Asia [16]. Hb E carriers in Thai (10-60%) and Burmese (11.4-60%) populations have been reported to vary markedly depending on the areas of investigation [17]. Results of the present study showed that Hb E is the most predominant form of thalassemia with prevalence rates of 2/12 (16.7%) and 17/89 (19.1%) in Thai and Burmese malaria patients, respectively. This was consistent with that previously observed in other Southeast Asian countries such as Laos and Cambodia [9]. The most severe form α -thalassemia-1, is found in Southeast Asia and China. In Thailand, the prevalence of this thalassemia type varies from 2.5 to 10% in each area of the country [9]. In addition, α -thalassemia-2 is also distributed in several malaria endemic countries including Nepal, India, and Papua New Guinea [18]. The common types are those with deletion of 3.7 and 4.2 kb. In Southeast Asia, the most common types of α -thalassemia-1 and α -thalassemia-2 are $--^{SEA}$ and $-\alpha^{3.7}$, respectively. The frequency of $-\alpha^{3.7}$ in Thai population in northeast

area of the country was reported as 17.5% [19].

The association between malaria disease severity and various red cell disorders were investigated in various studies [20-22]. Most studies were performed in patients with *P. falciparum* infection to explore the protective effect of red cell disorders on malaria. These red cell disorders included disorders related to merozoite invasion protection, parasite growth within the red blood cell, and the ability to eliminate malaria from red blood cells [23]. Study on the relationship between red cell disorders and the incidence of malaria in Myanmar suggested protection of thalassaemic red cells against severe falciparum malaria [21]. The mean parasitemia in α - or β -thalassaemia trait patients was significantly lower than patients with normal hemoglobin or heterozygous HbE. In a study in Thailand, HbA₂ and Hb E levels were shown not to be enhanced by *P. falciparum* infection. This suggests that Hb E disorder might be due to the natural selection of malaria [24]. Furthermore, it was shown that the Hb E trait red blood cells were resistant to invasion by *P. falciparum*, with unidentified membrane abnormality [25].

In this study, hemoglobin and hematocrit of patients with thalassemia other than Hb E were found to be significantly lower than those with normal red cells. However, no such relationship was found with those carrying Hb E. Results may at least in part support the assumption that thalassaemic red cells may protect patients from further development to severe malaria as a result of hyperparasitemia. Interestingly, it is of note for the coexistence of malaria with only α -thalassemia-2 but not α -thalassemia-1, the commonly found thalassemia type in Southeast Asia. It is possible that α -thalassemia-1 protects host red cells from malaria infection in this population. Definite conclusion on this issue should be further explored with inclusion of a larger sample in the analysis.

In conclusion, a trend of decreasing parasitemia was found

in malaria patients with coexistence of thalassemia. The results may, at least in part, support the protective effect of thalassemia on the development of hyperparasitemia and severe anemia in malaria patients. Nevertheless, definite conclusion on such relationship including the underlying mechanisms needs to be confirmed in a larger study.

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CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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