Original Article

Prevalence of α-Thalassemia in Malaria Patients in the Western Border of Thailand

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Abstract: *Malaria remains a major public health problem in Thailand particularly in relation to infections caused by Plasmodium falciparum and Plasmodium vivax. Thalassemia is also commonly found in malaria endemic areas. In Thailand, the common α-thalassemia variants reported are α-thalassemia-1 (SEA and THAI deletion) and α-thalassemia-2 (3.7 and 4.2 deletion). This study was conducted in 2013 to investigate the prevalence of α-thalassemia in a total of 71 malaria patients residing along the Thai-Myanmar border at Mae Sot district, Tak province, Thailand. The four common α-thalassemia variants were amplified using multiplex polymerase chain reaction (PCR). Only the 3.7 kb deletion α-thalassemia-2 was found in the samples with prevalence of 19.7%. Results from this preliminary study may indirectly imply the protective effect of α-thalassemia-1 against malaria infection.*

Keywords: ● *Malaria* ● *Thalassemia* ● *α-thalassemia*

RTA Med J 2014;67:149-54.

Introduction

Malaria is one of the parasitic diseases which remain a major public health problem in several tropical countries including Thailand. The infections caused by *Plasmodium falciparum* and *Plasmodium vivax* constitute a major burden leading to high morbidity and mortality in populations in several endemic areas. In Thailand, malaria spreads throughout the country particularly along the international borders, with the highest incidence in Tak province, in the western border of the country[1]. Thalassemia is the hemoglobinopathy that is also widely distributed in most malaria endemic areas[2]. This genetic disease is a result of the alteration in the globin chain of hemoglobin, a tetramer molecule consisting of two each of α- and β-globin chains. The α-thalassemia leads to a decrease in the synthesis of one or both of the α-globin chains on the chromosome 16p13.3[3]. This α-globin gene deletion is associated with the development of a mild form α-thalassemia-2 (-α/αα,-α/-α) or a severe form α-thalassemia-1 (--/αα,--/--). The prevalence of α-thalassemia is relatively high in several malaria endemic areas including the Mediterranean, Southeast Asia, Africa, and the Indian subcontinent[4]. Knowledge on the prevalence of α-thalassemia and its protective effect on malaria disease development, progression, and severity would be useful in providing guidance to malaria control policy. To date, the prevalence of

Received 19 November 2014 Accepted 18 December 2014

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α-thalassemia including its association with malaria infection in Thailand has not been reported. The aim of this preliminary study was to investigate the prevalence of α-thalassemia in malaria patients in Mae Sot district, Tak province on the western border of Thailand.

Materials and Methods

Study subjects and sample collection

A total of 71 blood samples were collected from patients (4 Thais and 67 Burmese) infected with *P. falciparum* (n = 26), *P. vivax* (n = 43), and mixed infection of both species (n = 2) who attended malaria clinics in Mae Sot district, Tak province, Thailand in 2013. Approval of the study protocol was obtained from the Ethics Committee of Thammasat University. Blood samples were stained with Giemsa and microscopically examined for the presence of malaria parasites.

Extraction of parasite genomic DNA

Parasite genomic DNA was extracted from whole blood using QIAamp DNA extraction mini-kit (QIAGEN, CA, USA) according to standard protocol and used as a template for polymerase chain reaction (PCR) amplification.

Amplification of α-thalassemia

Mutations of six variants of the genes encoded α-thalassemia were amplified using three sets of multiplex PCR[5-7]. These included SEA and THAI variants of α-thalassemia-1, the 3.7 and 4.2 kb deletion variants of α-thalassemia-2, Hb Constant Spring (HbCS), and Hb Pakse (HbPS).

Identification of α-thalassemia-1 with SEA (Southeast Asia) and THAI deletions was performed using T1 (5'-TGACTGCATCATAATTCCAGCAG-3') and T2 (5'-TGAGGCAGGAGATTCGCTTGA-3') primers. The three primers A7 (5'-CTCTGTGTTCTC AGTATTGGAG-3'), A9 (5'-ATATATGGGTCTGGAAGTGTATC-3'), and A1B (5'-GG TTCCCTGAGCCCC GACACG-3') were used to amplify the SEA deletion (660 bp) and internal control (314 bp)[5,6]. The PCR reaction mixture contained 50-100 ng DNA, 75 mM Tris-HCl (pH 8.0), 2 mM $MgCl_2$, 50 mM KCl, 20 μM $(NH_4)_2SO_4$, 200 mM of each dNTP, 67.5 pmol of A7 primer, 33.8 pmol of A9 primer, 2 pmol of A1B primer, 6 pmol of T1 and T2 primers, 1 M betaine, 1% DMSO, and 2.5 units Taq polymerase (Promega Co., Madison, USA). The PCR condition consisted of 1 cycle of 94°C for 3 min, 35 cycles of 94°C for 40 s, 61°C for 20 s, 72°C for 2 min, and final extension at 72°C for 2 min. The products were separated on a 2% agarose gel containing ethidium bromide.

The identification of α-thalassemia-2 with 3.7 and 4.2 kb deletions was performed using three sets of primers, *i.e.,* αG26 (5′-CTCAACAGATGCCAGCCAAA CAGAC-3′)/αG27 (5′-GAACCATTACACCAAGTGAA GG ACG-3′), A (5′-CCCAGAGCCAGGTTTGTTTATC TGT-3′)/B (5′-GAGGCCCAAGGGGCAAGAAGCAT-3′), and C (5′-GCTAGAGCATTGGTGGTCATGCC-3′)/D (5′-TTCTGACTCTGCCCACAGCCTGA-3′), to amplify the internal control (933 bp), 3.7 kb deletion (1,779 bp), and 4.2 kb deletion (1,529 bp), respectively[7]. The PCR reaction mixture contained 0.1 μg DNA, 30 pmol each of the A, B, C, and D primers, 15 pmol each of the αG26 and αG27 primers, 200 μM dNTPs, 75 mM Tris–HCl (pH 8.0), 50 mM KCl, 2 mM $MgCl_2$, 1 M betaine, 5% dimethylsulfoxide, and 2 units of Taq DNA polymerase (New England Biolabs Inc., MA, USA). The PCR condition consisted of 1 cycle of 94°C for 3 min, 10 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 2 min, and 20 cycles with 20 s increments in the extension step of each cycle. The products were separated on a 1.5% agarose gel containing ethidium bromide.

The identification of HbCS and HbPS mutations was performed using αG2 (5'GCTGACCTCCAAATACCGTC-3')/C3 (5'CCATTGTTGGCACATTCCGG-3'), and αG17 (5' AGATGGCGCCTTCCTCTCAGG-3')/αG18 (5'-ACGGCT ACCGAGGCTCCAGCA-3'), to amplify α^{CS} and α^{PS}

mutations, respectively[8]. The PCR reaction mixture contained 0.1 μg DNA, 37.5 pmol of αG2 primer, 30 pmol of C3 primer, 3.75 pmol of αG17 primer, 30 pmol αG18 primer, 200 μM dNTPs, and 2 units of Taq DNA polymerase (Promega Co., Madison, USA) in 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, 3.0 mM $MgCl_2$, 1 M betaine, and 4.8% dimethylsulfoxide. The PCR condition consisted of 1 cycle of 94°C for 30 min, 30 cycles of 94°C for 1 min, and 65°C for 1 min. The products were separated on a 1.5% agarose gel containing ethidium bromide.

Statistical analysis

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

Results

The prevalence of α-thalassemia in 71 patients with malaria infection in Mae Sot district, Tak province, Thailand was 19.7%. Four types of gene mutations were observed, *i.e.*, homozygous α-thalassemia-2, homozygous α-thalassemia-2 with Hb E, heterozygous α-thalassemia-2, and heterozygous α-thalassemia-2 with β-thalassemia (Table 1). The major types were heterozygous α-thalassemia-2 with and without β-thalassemia (16.9%). All patients with α-thalassemia-2 had a 3.7 kb gene deletion. The α-thalassemia-1 was not detected in any sample. The hematological parameters including hemoglobin, hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) are presented in Table 1. There was no significant association between α-thalassemia types and malaria species, patient gender, hemoglobin, and hematocrit values. The median values of MCV and MCH of patients with heterozygous α-thalassemia-2

Table 1 Prevalence of α-thalassemia 2, malaria species infected, patient gender, and hematological parameters in 71 malaria patients. The hematological parameters are presented as median (minimum-maximum) values.

Genotype of thalassemia	Number (%)	Malaria Type			Gender		Hb (g/dL)		Hct (%)		MCV (fl)		MCH (pg)	
		PF	PV	MIX	Male	Female								
Homozygous α-thal2	1 (1.4)	1	0	0	1	0	12.0	(12.0-12.0)	34.3	(34.3-34.3)	65.0	(65.0-65.0)	22.9	(22.9-22.9)
Homozygous α-thal2 with HbE	1 (1.4)	0	1	0	1	0	7.6	(7.6-7.6)	24.0	(24.0-24.0)	62.0	(62.0-62.0)[c]	19.7	(19.7-19.7)
Heterozygous α-thal2	9 (12.7)	2	7	0	5	4	12.0	(5.1-14.3)	36.5	(15.9-44.1)	74.0	(53.0-91.0)	24.1	(15.2-30.2)
Heterozygous α-thal2 with β-thal	3 (4.2)	1	2	0	1	2	10.9	(9.8-11.1)	33.6	(28.3-34.3)	67.0	(60.0-67.0)[a]	21.3	(19.6-22.7)[b]
Malaria without α-thalassemia	57 (80.3)	22	33	2	39	18	12.2	(3.4-16.4)	37.1	(10.7-49.1)	80.0	(50.0-101.0)	26.8	(18.7-32.3)

PF = *P. falciparum*; PV = *P. vivax*; Hb = hemoglobin; Hct = hematocrit; thal = thalassemia

[a] *Statistically significant difference from patients without thalassemia (p = 0.005)*

[b] *Statistically significant difference from patients without thalassemia (p = 0.001)*

[c] *Statistically significant difference from patients without thalassemia (p = 0.013)*

combined with β-thalassemia were significantly lower than those without thalassemia (MCV $p = 0.005$; MCH $p = 0.001$). Furthermore, the median MCV of patients with homozygous α-thalassemia-2 combined with Hb E was significantly lower than patients without thlassemia ($p = 0.013$).

Discussion and Conclusion

The α-Thalassemia is commonly found in Southeast Asia and China, and the most common variants of which are α-thalassemia-1–^{SEA} and α-thalassemia-2-$\alpha^{3,7}$. In Thailand, the prevalence of α-thalassemia in different regions of the country varies from 2.5 to 30%[9,10]. Results of the present study revealed only the 3.7 kb deletion α-thalassemia-2 in the western region of the country at frequency of 19.7%. The prevalence of α-thalassemia in the population in the western region of Thailand has not been previously reported. This frequency is similar to what was reported in the population in the northeastern region of the country (17.5%)[11]. As most of the study population was Burmese, this observed prevalence could have been confounded by influence of ethnicity. An average prevalence of 5-37.5% of α-thalassemia has been reported in Burmese population[12,13]. The most common α-thalassemia variant (29.7%) observed in the Shan State close to the northwest border of Thailand is heterozygous α-thalassemia-2[13].

The α-thalassemic red cells were reported to protect the malaria infected host against disease development and progression[14,15]. This protective effect was supported by the reports from Tanzania and Kenya[16,17], Vanuatu[18], and Ghana[19]. The proposed mechanism is the reduction of the ability of the infected thalassemic red cells to form rosettes with the uninfected red cells and thus preventing the sequestration of malaria parasites in blood capillaries[20]. In addition, thalassemic red cells increase the amounts of immunoglobulin, immune recognition, resulting in the acceleration of the clearance of infected red cells[21]. The α-thalassemic red cells also promote the pro-inflammatory effect of cytoadherence[22]. The α-Thalassemia-2 does not appear to protect malarial infection including progression to symptomatic or high parasitemia, but reduce the risk of developing severe malaria[15]. The study in Melanesian children showed the lack of protective effect of α-thalassemia-2 against uncomplicated *P. falciparum*[23]. The observation of only α-thalassemia-2 in this patient population may indirectly imply the protective effect of α-thalassemia-1 against malaria infection. However, the limitation of the current study is the lack of non-malaria patient population, to investigate the relationship between malaria and thalassemia. Further study with increasing sample size is needed to confirm the protective effect of α-thalassemia on malaria.

Acknowledgements

The study was supported by Thammasat University (TU) Research Fund under the TU Research Scholar Contract No. 57/2555. KN was supported by the Commission on Higher Education (National University Project), Ministry of Education, and Thammasat University (Center of Excellence in Pharmacology and Molecular Biology of Malaria and Cholangiocarcinoma).

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ความชุกของแอลฟ่าธาลัสซีเมีย ในผู้ป่วยมาลาเรียภาคตะวันตกของประเทศไทย

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บทคัดย่อ *มาลาเรียปัญหายังคงปัญหาสาธารณสุขที่สำคัญในประเทศไทยโดยเฉพาะมาลาเรียชนิดฟัลซิพารัมและไวแวกซ์ นอกจากนี้ในพื้นที่ระบาดของมาลาเรียมักพบอุบัติการณ์ของธาลัสซีเมียด้วย โดยแอลฟ่าธาลัสซีเมียชนิดที่พบบ่อยคือแอลฟ่าธาลัสซีเมีย-1 และ -2 การศึกษานี้ได้ดำเนินการในปี พ.ศ. 2556 โดยมีวัตถุประสงค์เพื่อศึกษาความชุกของแอลฟ่าธาลัสซีเมียในผู้ป่วยมาลาเรียที่อาศัยในพื้นที่ อำเภอแม่สอด จังหวัดตาก จำนวน 71 ราย โดยศึกษาแอลฟ่าธาลัสซีเมียชนิดที่พบบ่อยคือ แอลฟ่าธาลัสซีเมีย-1 (SEA และ THAI deletion) และแอลฟ่าธาลัสซีเมีย-2 (3.7 และ 4.2 deletion) ด้วยวิธี multiplex polymerase chain reaction (PCR) ในประชากรที่ศึกษาพบเฉพะแอลฟ่าธาลัสซีเมีย-2 ชนิด 3.7 deletion เพียงชนิดเดียว ผลการศึกษาในเบื้องต้นนี้อาจแสดงให้เห็นถึงผลในการป้องกันการติดเชื้อมาลาเรียทั้งชนิดฟัลซิพารัมและชนิดไวแวกซ์ของแอลฟ่าธาลัสซีเมีย-1 ในผู้ป่วยมาลาเรียที่อาศัยในพื้นที่นี้*

Keywords: ● *มาลาเรีย* ● *ธาลัสซีเมีย* ● *แอลฟ่าธาลัสซีเมีย*

เวชสารแพทย์ทหารบก 2557;67:149-54.