PREVALENCE AND CHARACTERIZATION OF THALASSEMIA AMONG MIGRANT WORKERS FROM CAMBODIA, LAO PDR AND MYANMAR IN THAILAND

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Abstract. Thalassemia is one of the most common inherited diseases worldwide and is considered as a major public health concern in many countries including Thailand. With the establishment of ASEAN Economic Community (AEC), thalassemia prevention and control in Thailand will be more difficult as more AEC workers migrate into the country. This study characterized prevalence of thalassemia among migrant workers from Cambodia, Lao PDR and Myanmar. Among 3,227 blood samples, 46.9% were thalassemia heterozygotes or disease, with 17 different thalassemia genotypes. Highest prevalence of homozygous (12.8%) and heterozygous (39.7%) Hb E were among workers from Lao PDR, while prevalence of α-thalassemia 1 carrier (1.8%), α-thalassemia 2 carrier (19.9%) and β-thalassemia heterozygote (3.9%) were highest among workers from Myanmar. These data should prove useful in formulating health policy for prevention, control and treatment of thalassemia among these migrant workers in Thailand.

Keywords: thalassemia, prevalence, migrant worker, Cambodia, Lao PDR, Myanmar,

INTRODUCTION

Hemoglobinopathies are the most common monogenic diseases worldwide, with approximately 7% of the world's population being carriers of thalassemias, arising from defective globin synthesis **(**Weatherall and Clegg, 2001). In Southeast Asia, gene frequency of α-thalassemia (defective α-globin synthesis) reach 20-30% in northern Thailand and Lao PDR, that of β-thalassemia (defective β-globin synthesis) 1-9%, that of Hemoglobin Constant Spring (Hb CS) 1-8%, and Hb E 13%, with up to 50% in the borders of Cambodia, Lao PDR and Thailand (Fucharoen and Winichagoon, 1992).

Pathologic features of red cells and anemia in these disorders are the consequences of excess unmatched globin chains. Accumulation and deposition of these unmatched globin chains in erythroid precursors cause premature death

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of these cells in bone marrow (resulting in ineffective erythropoiesis), and deposition of these molecules in circulating thalassemia red blood cells results in their premature clearance by the body reticuloendothelial system (known as hemolytic anemia) (Schrier, 2002). Generally, thalassemia heterozygotes, in particular, carriers of α-thalassemia 1 and β-thalassemia carriers, are symptomless but with a typical characteristic of microcytic red blood cells (Fucharoen and Winichagoon, 1989). Mean corpuscular volume (MCV) < 80 fl and mean corpuscular hemoglobin (MCH) <27 pg are usually used as indicators for further analysis of the presence of thalassemia carriers. Diagnosis of α- and β-thalassemia syndromes are based on family data and Hb typing, which can be quantified by high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) (Fucharoen *et al*, 1998; Winichagoon *et al*, 2008). Subjects heterozygous for α- or β-thalassemia have the same Hb type, A_2A, but the amount of Hb A_2 is 4-6% in β-thalassemia heterozygote. Hb E heterozygote is completely symptomless but the homozygous state is characterized by microcytosis similar in magnitude to that of β-thalassemia trait (Tatsumi *et al*, 1989). In some populations of Southeast Asia, diagnosis using the above protocols can be uncertain due to the heterogeneity of thalassemia genotypes, viz. co-inheritance α-thalassemia with β-thalassemia may normalize hematological values (Yamsri *et al*, 2011). Definitive diagnosis for α-thalassemia trait is generally confirmed by PCR-based techniques (Winichagoon *et al*, 1995; Tang *et al*, 2001).

Migrant workers in Thailand are increasing every year, particularly from Cambodia, Lao PDR and Myanmar; in 2014, the number was 1.37 million, an increase of nearly 200,000 from the previous year (Ministry of Labor, 2017). This could rise even higher when Thailand joined the ASEAN Economic Community (AEC) in 2015. These migrant workers will bring with them thalassemia genes and consequently couples at risk of conceiving children with thalassemic disease. Screening and providing genetic counseling for such couples will require knowledge of the molecular basis of their thalassemia genes. Thus, there is an urgent need to obtain information on the prevalence of the major forms of thalassemia among these migrant workers.

In this study prevalence of molecular types of thalassemia genes among the 3,227 migrant workers in Thailand were assessed using hematologic parameters, Hb and DNA analyses. It is expected that these data will enable the Thai authority to issue an appropriate policy for thalassemia screening among migrant workers from neighboring countries where thalassemia is prevalent.

MATERIALS AND METHODS

The protocol was approved by the Mahidol University Institution Review Board, Mahidol University, Bangkok, COE No. 2014/001.0702, and was carried out following the rules of the Declaration of Helsinki (1975). A total of 3,227 blood samples were obtained from migrant workers from Cambodia, Lao PDR and Myanmar at the Thalassemia Research Center, Mahidol University and anonymously encoded, recording only the donor nationality. Three ml of venous blood sample were drawn into EDTA tube for hematological and HPLC analyses, and a portion used for DNA analysis. Complete blood counts (CBC) were performed using a Sysmex XT-2000i automated hematology

analyzer (Sysmex Corporation, Chuo-ku, Kobe, Japan) within 1-3 hours after blood collection. The instrument calibrator was used for calibration, and three instrument controls for low, normal and high values were routinely examined prior to each run.

Diagnosis of α- and β-thalassemia was based on Hb type and quantity obtained from an automated HPLC instrument (VARIANT™, Bio-Rad, Hercules, CA). Confirmation for α-thalassemia 1 and α-thalassemia 2 genes, and genotyping of β-thalassemia were performed by PCR-based techniques, namely, gap-PCR for α-thalassemia (Winichagoon *et al*, 1995; Tang *et al*, 2001) and reverse dot-blot (RDB) hybridization for β-thalassemia genotypes (Winichagoon *et al*, 1999).

Results are presented as mean ± SD. Data was analyzed using SPSS software version 18.0 (IBM, Armonk, NY) and a p-value < 0.05 is considered significant.

RESULTS

Prevalence of thalassemias among migrant workers from Cambodia, Lao PDR and Myanmar in Thailand

Based on DNA analysis of 1,438 samples from migrant workers from Cambodia, Lao PDR and Myanmar, the three major defective globin genes were α-thalassemia (both α-thalassemia 1 and α-thalassemia 2), β-thalassemia and Hb E accounting for 46.9% of the samples (Table 1). As expected, Hb E allele is most prevalent among those from Cambodia and Lao PDR, while α-thalassemia and β-thalassemia are more common among workers from Myanmar. The overall prevalence of Hb E allele in this study was 0.217: 80/762 (0.105) from Myanmar, 103/312 (0.33) from Lao PDR and 441/1802 (0.245) from Cambodia. Among workers from Myanmar, prevalence of α-thalassemia 1 and α-thalassemia 2 alleles was 0.012 and 0.115, respectively, while that of β-thalassemia allele was 0.021, higher compared with prevalence among migrant workers from Cambodia (0.007, 0.073 and 0.003, respectively) and Lao PDR (0.010, 0.087 and 0.013, respectively). Because of the high prevalence of both α-thalassemia and Hb E, heterozygous Hb E combined with α-thalassemia was detected as well (Table 1). Thalassemia diseases identified among these migrant workers included 6 patients with Hb H disease and 1 EABart's disease in migrant workers from Cambodia and 1 β-thalassemia/Hb E each from Cambodia and Lao PDR, accounting for 0.6% of 1,438 samples.

Hematological profiles and Hb analysis in thalassemia syndromes

Hematological parameters and Hb analysis were analyzed in conjunction with thalassemia genotyping. Subjects with Hb E have Hb E+A (heterozygotes) while thalassemia carriers Hb A_2+A. Although α-thalassemia 1 and β-thalassemia heterozygotes have similar values of red cell indices, they are distinguished by an increased percent Hb A_2 level (5.2 ± 0.7) in β-thalassemia heterozygotes (Table 2). Subjects with β-thalassemia disease (homozygous β-thalassemia and β-thalassemia/Hb E) and Hb H disease were diagnosed by their clinical features (Nienhuis and Nathan, 2012; Vichinsky, 2013) and Hb typing: homozygous β-thalassemia has Hb A2+F or A2+F+A; β-thalassemia/Hb E has Hb E+F, and Hb H disease has Hb A2+A+H or Hb CS+A2+A+H.

Red cell parameters of many subjects with α-, β-thalassemia and Hb E are clearly different from normal (Table 2). However, it was almost impossible to distinguish α-thalassemia 2 heterozygotes from normal individuals, although MCV, MCH and red

Table 1
Prevalence of thalassemia in migrant workers in Thailand.

Phenotype	Genotype	Migrant worker		
		Myanmar Number (%)	Lao PDR Number (%)	Cambodia Number (%)
Normal	$\alpha\alpha/\alpha\alpha,\beta^A\beta^A$	220 (58)	51 (33)	410 (45.5)
Heterozygous α-thalassemia 1				
$--^{SEA}$ deletion	$--^{SEA}/\alpha\alpha,\beta^A\beta^A$	5 (1)	1 (1)	4 (0.4)
$--^{THAI}$ deletion	$--^{THAI}/\alpha\alpha,\beta^A\beta^A$	0 (0)	1 (1)	0 (0)
Heterozygous α-thalassemia 2				
$-\alpha^{3.7}$ kb deletion	$-\alpha^{3.7}/\alpha\alpha,\beta^A\beta^A$	58 (15)	15 (10)	81 (9.0)
$-\alpha^{4.2}$ kb deletion	$-\alpha^{4.2}/\alpha\alpha,\beta^A\beta^A$	2 (0.5)	0 (0)	1 (0.1)
Homozygous α-thalassemia 2				
$-\alpha^{3.7}$ kb deletion	$-\alpha^{3.7}/-\alpha^{3.7}$, $\beta^A\beta^A$	3 (1)	1 (1)	2 (0.2)
Hb H disease	$--^{SEA}/-\alpha^{3.7}$, $\beta^A\beta^A$	2 (0.5)	1 (1)	3 (0.3)
Heterozygous Hb E	$\alpha\alpha/\alpha\alpha$, $\beta^E\beta^A$	53 (14)	56 (36)	310 (34.4)
Heterozygous Hb E combined with α-thalassemia 1				
$--^{SEA}$ deletion	$--^{SEA}/\alpha\alpha$, $\beta^E\beta^A$	2 (0.5)	0 (0)	4 (0.4)
$--^{THAI}$ deletion	$--^{THAI}/\alpha\alpha$, $\beta^E\beta^A$	0 (0)	0 (0)	0 (0)
Heterozygous Hb E combined with α-thalassemia 2				
$-\alpha^{3.7}$ kb deletion	$-\alpha^{3.7}/\alpha\alpha$, $\beta^E\beta^A$	13 (3)	4 (3)	29 (3.2)
$-\alpha^{4.2}$ kb deletion	$-\alpha^{4.2}/\alpha\alpha$, $\beta^E\beta^A$	0 (0)	0 (0)	1 (0.1)
Heterozygous Hb E combined with homozygous α-thalassemia 2	$-\alpha^{3.7}/-\alpha^{3.7}$, $\beta^E\beta^A$	2 (0.5)	1 (1)	3 (0.3)
Homozygous Hb E	$\alpha\alpha/\alpha\alpha$, $\beta^E\beta^E$	4 (1)	16 (10)	40 (4.4)
Homozygous Hb E combined with heterozygous α-thalassemia 2				
$-\alpha^{3.7}$ kb deletion	$-\alpha^{3.7}/\alpha\alpha$, $\beta^E\beta^E$	1 (<0.5)	3 (2)	5 (0.5)
$-\alpha^{4.2}$ kb deletion	$-\alpha^{4.2}/\alpha\alpha$, $\beta^E\beta^E$	0 (0)	1 (1)	1 (0.1)
Heterozygous β-thalassemia	$\alpha\alpha/\alpha\alpha$, $\beta^{Thal}\beta^A$	13 (3)	2 (1)	5 (0.5)
Heterozygous β-thalassemia combined with α-thalassemia 2				
$-\alpha^{3.7}$ kb deletion	$-\alpha^{3.7}/\alpha\alpha$, $\beta^{Thal}\beta^A$	2 (0.5)	0 (0)	0 (0)
$-\alpha^{4.2}$ kb deletion	$-\alpha^{4.2}/\alpha\alpha$, $\beta^{Thal}\beta^A$	0 (0)	0 (0)	0 (0)
Hereditary persistence of Hb F	$\alpha\alpha/\alpha\alpha$, $\beta^{HPFH}\beta^A$	0 (0)	1 (1)	0 (0)
β-thalassemia/Hb E	$\alpha\alpha/\alpha\alpha$, $\beta^{Thal}\beta^E$	0 (0)	1 (1)	1 (0.1)
EABart's disease	$--^{SEA}/-\alpha^{3.7}$, $\beta^E\beta^A$	0 (0)	0 (0)	1 (0.1)
Hb variant				
- α-globin variant combined with heterozygous Hb E	$\alpha^T\alpha/\alpha\alpha$, $\beta^E\beta^A$	0 (0)	1 (1)	0 (0)
- β-globin variant	$\alpha\alpha/\alpha\alpha$, $\beta^T\beta^A$	1 (<0.5)	0 (0)	0 (0)
Total		381 (100)	156 (100)	901 (100)

blood cell distribution width (RDW) are significantly different (p <0.01) (Fig 1). Red blood cell morphology of α-thalassemia 2 heterozygote (who do not have anemia) are normal. Although their RDWs are significantly different, the values of 13.5 ± 1.2 in normal individuals and 14.6 ± 1.3 in α-thalassemia 2 heterozygotes are not significantly different (Table 2).

Homozygous α-thalassemia 2 are clinically normal and have similar values of MCV/MCH and RDW; hence red cell indices of homozygous α-thalassemia 2 differ significantly from α-thalassemia

Table 2
Red cell indices and hemoglobin analysis of each thalassemia genotype in 3,227 migrant workers from Cambodia, Lao PDR and Myanmar in Thailand.

Phenotype	Genotype	Number	Red cell index					Hb analysis		
			Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	Hb pattern	Hb A_2/E (%)	Hb F (%)
Normal	αα/αα,$\beta^A\beta^A$	1,857	14.3 ± 1.4	88.0 ± 4.9	28.9 ± 1.9	32.9 ± 1.0	13.5 ± 1.2	A_2A	2.9 ± 3.2	0.5 ± 0.4
Heterozygous α-thalassemia 1	--/αα,$\beta^A\beta^A$	17	13.4 ± 0.9	66.0 ± 5.7	21.2 ± 1.9	32.0 ± 0.5	17.9 ± 1.6	A_2A	2.4 ± 0.3	0.6 ± 0.4
Heterozygous α-thalassemia 2	-α/αα,$\beta^A\beta^A$	201	13.7 ± 1.4	80.0 ± 5.0	25.9 ± 1.8	32.3 ± 0.8	14.6 ± 1.3	A_2A	3.2 ± 4.1	0.5 ± 0.6
Homozygous α-thalassemia 2	-α/-α, $\beta^A\beta^A$	16	14.3 ± 0.8	69.5 ± 2.7	21.7 ± 0.9	31.3 ± 0.9	16.9 ± 2.3	A_2A	2.6 ± 0.4	0.2 ± 0.1
Hb H disease	--/-α, $\beta^A\beta^A$	11	10.2 ± 1.4	61.5 ± 8.4	18.3 ± 2.1	30.0 ± 1.4	22.7 ± 3.4	A_2A Bart'sH	1.4 ± 0.5	0.9 ± 0.5
Heterozygous Hb E	αα/αα, $\beta^E\beta^A$	826	13.7 ± 1.3	77.6 ± 3.9	25.6 ± 1.4	33.0 ± 0.7	14.6 ± 1.1	EA	27.6 ± 2.2	1.0 ± 1.0
Heterozygous for Hb E combined with α-thalassemia 1	--/αα, $\beta^E\beta^A$	7	13.1 ± 1.6	65.5 ± 2.3	21.3 ± 0.8	32.6 ± 0.5	17.2 ± 1.7	EA	19.1 ± 1.3	0.8 ± 0.5
Heterozygous Hb E combined with α-thalassemia 2	-α/αα, $\beta^E\beta^A$	78	13.5 ± 1.4	78.0 ± 5.0	25.7 ± 1.7	33.0 ± 0.8	14.8 ± 1.3	EA	25.0 ± 2.4	1.0 ± 1.0
Heterozygous Hb E combined with homozygous α-thalassemia 2	-α/-α, $\beta^E\beta^A$	17	13.3 ± 1.1	71.6 ± 2.8	23.3 ± 1.6	32.5 ± 0.9	15.0 ± 1.1	EA	20.1 ± 1.9	0.8 ± 0.4
Homozygous Hb E	αα/αα, $\beta^E\beta^E$	129	12.3 ± 1.3	56.7 ± 2.9	19.6 ± 1.1	34.7 ± 0.7	18.8 ± 1.6	EE	79.4 ± 9.4	3.8 ± 2.6
Homozygous Hb E combined with heterozygous α-thalassemia 2	-α/αα, $\beta^E\beta^E$	16	13.3 ± 1.4	60.6 ± 4.2	21.0 ± 1.3	34.7 ± 0.6	18.1 ± 1.8	EE	81.2 ± 9.0	2.7 ± 1.3
Heterozygous β-thalassemia	αα/αα, $\beta^{Thal}\beta^A$	32	12.1 ± 1.4	64.0 ± 7.5	20.7 ± 2.4	32.3 ± 0.8	17.9 ± 2.4	A_2A	5.2 ± 0.7	1.3 ± 1.0
Heterozygous β-thalassemia combined with α-thalassemia 2	-α/αα, $\beta^{Thal}\beta^A$	3	12.7 ± 0.8	65.8 ± 6.1	21.1 ± 2.2	32.0 ± 0.2	16.4 ± 3.2	A_2A	5.1 ± 0.2	1.4 ± 0.7
Hereditary persistence of Hb F	αα/αα, $\beta^{HPFH}\beta^A$	1	11.0	68.7	21.5	31.3	20.7	A_2FA	2.4	15.4
β-thlassemia/Hb E	αα/αα, $\beta^{Thal}\beta^E$	4	9.9 ± 2.1	59.3 ± 4.6	19.4 ± 2.4	32.6 ± 1.6	24.8 ± 5.0	EF	55.3 ± 11.1	27.6 ± 21.0
EABart's disease	$--^{SEA}$/$-\alpha^{3.7}$, $\beta^E\beta^A$	3	9.6 ± 1.1	48.4 ± 3.6	16.2 ± 3.8	33.6 ± 3.9	21.7 ± 1.2	EA Bart'sH	14.1 ± 0.3	1.6 ± 1.6
Hb variant										
- α-globin variant	$\alpha^T\alpha$/αα, $\beta^A\beta^A$	1	17.0	83.7	29.2	34.8	13.0	A_2A Ab Hb*	2.2	1.7
- α-globin variant combined with heterozygous β-thalassemia	$\alpha^T\alpha$/αα, $\beta^{Thal}\beta^A$	1	16.6	76.7	26.3	34.2	14.7	A_2A Ab Hb	4.0	0
- α-globin variant combined with heterozygous Hb E	$\alpha^T\alpha$/αα, $\beta^E\beta^A$	6	12.8 ± 1.5	75.2 ± 9.9	27.7 ± 2.8	32.9 ± 0.7	15.7 ± 3.1	EA Ab Hb	26.4 ± 10.1	2.8 ± 3.1
- β globin variant	αα/αα, $\beta^T\beta^A$	1	14.6	75.7	25.6	33.8	15.1	A_2A Ab Hb	2.4	0.4
Total		3,227								

*Ab Hb, Abnormal hemoglobin or Hb variant.

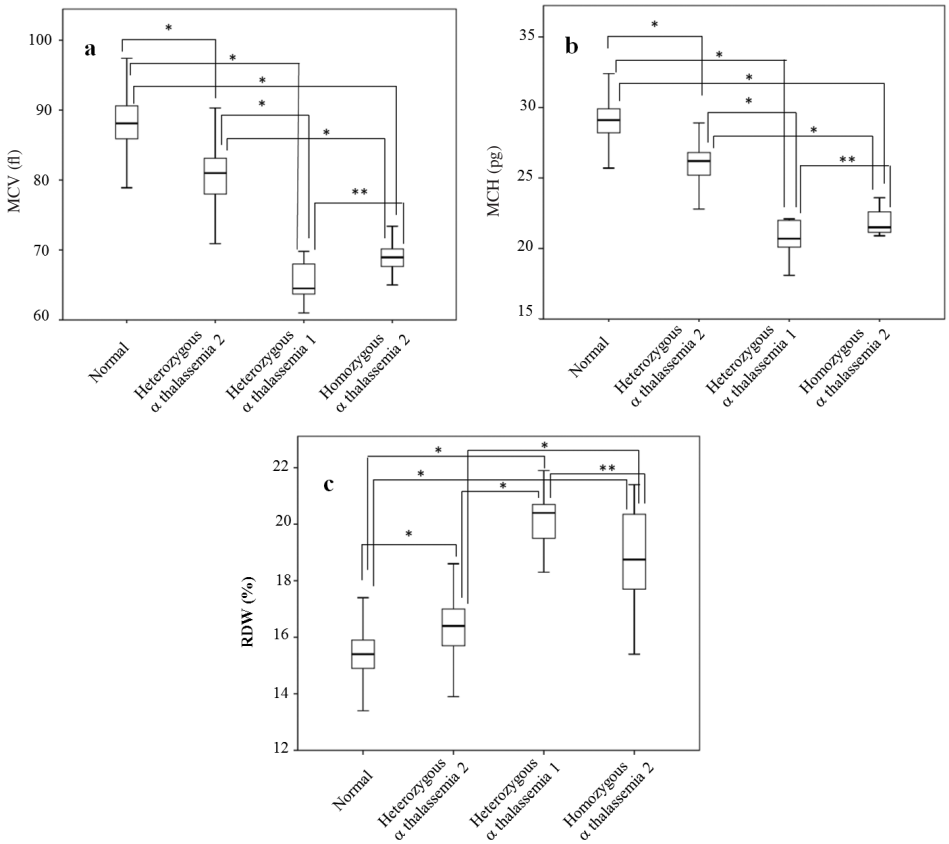

Fig 1–Red cell MCV (a), MCH (b) and RDW (c) compared among normal, heterozygous α-thalassemia 2, heterozygous α-thalassemia 1 and homozygous α-thalassemia 2 migrant workers from Cambodia, Lao PDR and Myanmar in Thailand. Red cell indices were measured using a Sysmex XT-2000i automated hematology analyzer (Sysmex Corporation, Chuo-ku, Kobe, Japan). *p <0.05; **p ≥0.05.

2 heterozygote and normal subjects (p <0.01) (Fig 1). The data in this study demonstrate that both α-thalassemia 1 heterozygotes and α-thalassemia 2 homozygotes have MCV values <76 fl and MCH levels < 23 pg (Table 2).

Hb E heterozygotes have MCV of 77.6 ± 3.9 fl and mean corpuscular hemoglobin concentration (MCHC) of 33 ± 0.7% (Table 2). Red cell parameters and percent Hb E in double heterozygous for Hb E and α-thalassemia 2 are also similar to those of Hb E heterozygotes. However, these parameters are significantly lower in double heterozygous for Hb E and α-thalassemia 1 with MCV of 65.5 ± 2.3 fl, MCH of 21.3 ± 0.8 pg and RDW of 17.2 ± 1.7 (p <0.01) (Fig 2). Percent Hb E is decreased to 19.1 ± 1.3 compared to 27.6 ± 2.2 and 25.0 ± 2.4 of Hb E heterozygote and double heterozygous for Hb E and α-thalassemia 2, respectively (Table 2). However, individuals with co-inheritance of heterozygous Hb E and homozygous α-thalassemia 2 have higher MCV (71.6 ± 2.8 fl) and lower RDW (14.6 ± 1.1) than those of double heterozygous for Hb E and α-thalassemia 1 (p <0.01) (Fig 2). Percent Hb E (20.1 ± 1.9) in heterozygous Hb E combined with homozygous

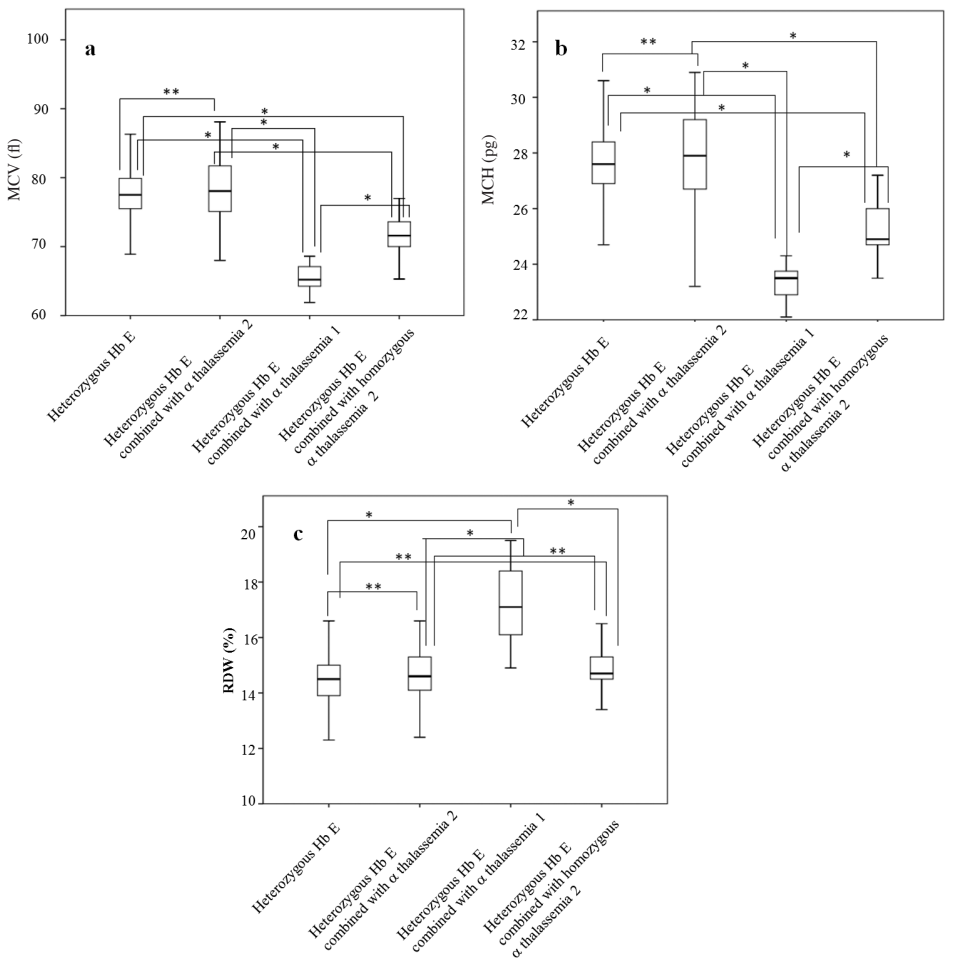

Fig 2–Red cell MCV (a), MCH (b) and RDW (c) compared among heterozygous Hb E, heterozygous Hb E combined with α-thalassemia 2, heterozygous Hb E combined with α-thalassemia 1 and heterozygous Hb E combined with homozygous α-thalassemia 2 migrant workers from Cambodia, Lao PDR and Myanmar in Thailand. Red cell indices were measured using a Sysmex XT-2000i automated hematology analyzer (Sysmex Corporation, Chuo-ku, Kobe, Japan). *p <0.05; **p ≥0.05.

α-thalassemia 2 is comparable to that of heterozygous Hb E combined with α-thalassemia 1.

Homozygous Hb E individuals do not develop clinical symptoms, but may have slight anemia but there is no jaundice or splenomegaly (Fucharoen and Weatherall, 2012). Diagnosis of Hb E homozygote is usually based on Hb type, with almost 100% Hb E. Red blood cell morphology present large numbers of microcytic red cells (MCV of 56.7 ± 2.9 fl) and also reduced MCH (19.6 ± 1.1 pg), which is in proportion with the reduced MCV value so that normal MCHC (34.7 ± 0.7 g/dl) I obtained (Table 2). Although MCV (60.5 ± 4.1 fl) and MCH (21.0 ± 1.3 pg) in a group of 16 subjects carrying homozygous Hb E together with heterozygous α-thalassemia 2 are significantly different from those of

Table 3
Cut-off values of MCV (85 fl) and MCH (27 pg) for differentiating among normal, α-thalassemia trait, heterozygous Hb E and heterozygous Hb E combined with α-thalassemia trait.

Phenotype	Number	MCV <85 fl Number (%)	MCV ≥85 fl Number (%)	MCH <27 pg Number (%)	MCH ≥27 pg Number (%)
Normal	1,857	231 (12.4)	1,626 (87.5)	154 (8.3)	1,703 (91.7)
Heterozygous α-thalassemia 2	201	187 (93)	15 (7)	155 (77)	46 (23)
Heterozygous α-thalassemia 1	17	17 (100)	0 (0)	17 (100)	0 (0)
Homozygous α-thalassemia 2	16	16 (100)	0 (0)	16 (100)	0 (0)
Heterozygous Hb E	826	801 (97.0)	25 (3.0)	708 (85.7)	118 (14.3)
Heterozygous Hb E combined with α-thalassemia 2	78	73 (94)	5 (6)	57 (73)	21 (27)
Heterozygous Hb E combined with α-thalassemia 1	7	7 (100)	0 (0)	7 (100)	0 (0)
Heterozygous Hb E combined with homozygous α-thalassemia 2	17	17 (100)	0 (0)	17 (100)	0 (0)

Hb E homozygotes (Table 3), it is generally difficult to detect co-inheritance of α-thalassemia in homozygous Hb E individuals.

All thalassemia diseases detected in the migrant workers had mild to moderate clinical symptoms and none required regular blood transfusion.

DISCUSSION

The thalassemias are a major public health problem in Southeast Asia, and can be an important contributor to the high prevalence of anemia than iron deficiency (Fucharoen and Winichagoon, 2011), Given the increase in numbers of migrant workers from Cambodia, Lao PDR and Myanmar in Thailand, determination of the potential burden of thalassemia disease in the country is important for implementation of a national prevention and control program. The best approach to cope with thalassemia in Southeast Asian countries is to prevent birth of cases with major thalassemic disease. A national program launched to prevent and control three major severe forms of thalassemic diseases, namely, homozygous β^0-thalassemia, β^0-thalassemia/Hb E and Hb Bart's hydrops fetalis, has produced encouraging results in many countries (Fucharoen and Weatherall, 2016). The burden of thalassemia disease is estimated by the prevalence of the disease-producing thalassemia alleles (Cao and Galanello, 2010). Hence genetic screening and provision of gene counseling to risk couples among migrant workers are required.

In Cambodia, Hb E is the most prevalent hemoglobinopathy, estimated to affect one-third of the population (Sanguansermsri *et al*, 1987), with prevalence of α-thalassemia of 35.4% and β-thalassemia 0.8% (Carnley *et al*, 2006). In Lao PDR a 40% prevalence of α-thalassemia trait (mostly α-thalassemia 2), 9% β-thalassemia and 40-50% Hb E have been reported (Sanguansermsri *et al*, 2004; Sengchanh *et al*, 2005; Sengchanh and Phengsavanh,

2012). In 2012, according to the country's reports of "The 1st Pan-Asian Conference on Hemoglobinopathies", Myanmar has 10-14% prevalence of α-thalassemia carrier, 0.8-1.7% β-thalassemia carrier and 10-30% Hb E carrier (Ne-Win, 2012). Similar results were observed in our study of migrant workers from these three neighboring countries.

In theory, premarital screening for at-risk couples and in pregnant women early in pregnancy should be able to reduce the incidence of thalassemia diseases. However, accurate diagnosis is still not available in many Southeast Asian countries due to difficulty in the identification of thalassemia traits. It is now possible to identify β-thalassemia and Hb E carriers with accuracy using automated high HPLC system (Stephens *et al*, 2015). Diagnosis of thalassemia traits is also possible based on the changes of red blood cell morphology (Fucharoen *et al*, 1989; Ryan *et al*, 2010). Automated Flow cytometry-based hematology analyzer has enabled heterogeneity of red cell parameters in α- and β-thalassemia carriers and diseases states to be rapidly identified (Kim and Ornstein, 1983; Tycko *et al*, 1985; Mohandas *et al*, 1986). Measurements of MCV and MCH are useful as a first screening step for thalassemia traits as MCV and MCH are decreased in the majority of thalassemia carriers (Fucharoen *et al*, 1989; Bunyaratvej *et al*, 1994; d'Onofrio *et al*, 1995). In addition, RDW varies greatly among different types of thalassemia. This study shows that an MCV value <85 fl and MCH <27 pg detect 93% and 77%, respectively of α-thalassemia 2 heterozygote. The above cut-off values of MCV and MCH can also detect all cases of heterozygous α-thalassemia 1 and homozygous α-thalassemia 2. The latter two α-thalassemia conditions can be distinguished from each other by DNA analysis only. Red cell parameters of heterozygous β-thalassemia are similar to those of heterozygous α-thalassemia 1 and homozygous α-thalassemia 2 and an increased percent Hb A_2 of 5.2 ± 0.7 was indicative for β-thalassemia trait. Nevertheless, genotyping for β^0- (severe form) and β^+- (mild form) thalassemia alleles is necessary for subsequent informative genetic counseling.

In Hb E subjects, differentiation between heterozygous and homozygous individuals is readily possible using Hb typing and red blood cell indices. However, the diagnosis can be problematic when there is concomitant inheritance of α-thalassemia. Red cell parameters in heterozygous Hb E combined with α-thalassemia 2 are not significantly different from those of Hb E heterozygote. The cut-off values of MCV at 85 fl and MCH at 27 pg were unable to distinguish between these two Hb E phenotypes. However, values below these cut-off values detected all cases of heterozygous Hb E combined with α-thalassemia 1 and individuals with co-inheritance of heterozygous Hb E and homozygous α-thalassemia 2. It should be noted that all heterozygous Hb E combined with α-thalassemia 1 individuals have MCV <70 fl and MCH <23 pg with RDW of 17.2 ± 1.7.

Thai thalassemia screening guidelines use an MCV <80 fl and/or MCH <27 pg as positive indicator(s) for further investigation for presence of α- or β-thalassemia allele (Sanchaisuriya *et al*, 2005). Our study also shows no false negative cases for α-thalassemia 1 and β-thalassemia heterozygotes when these cut-off values were used.

In conclusion, our data confirm the urgent need for health policy on thalassemia prevention and control among

migrant workers from countries where carriers of this inherited blood disorder are prevalent.

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