Original Article

Multiplex ARMS-PCR Analysis for Nineteen β-Thalassemia Mutations

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Abstract : *β-Thalassemia is a heterogeneous group of inherited hematological disorders. In Thailand, gene frequency of β-thalassemia varies between 3-9%. We have designed 4 sets of primers to use in multiplex amplification refractory mutation system polymerase chain reaction (MARMS-PCR) to detect 19 β-thalassemia mutations in 250 β-thalassemia samples including 178 heterozygous β-thalassemia, 2 homozygous β-thalassemia, and 70 β-thalassemia/Hb E. Fourteen different mutations were identified, of which the five most common (codon 41/42 (-TTCT), codon 17 (A>T), nt-28 (A>G), IVS II-654 (C>T) and IVS I-5 (G>C)) accounted for 83%. In heterozygous β-thalassemia, coexistence of α-thalassemia, thereby producing a more balanced α- to β-globin synthesis ratio, raised Hb concentration as well as MCV and MCH. On the other hand, a decrease in MCV and MCH in β^0-thalassemia/HbE with coinheritance of 3.7-kb deletion α^+-thalassemia was presumed to be related to a decrease in Hb F and an increase in Hb E levels.*

Key Words : ● *Thalassemia* ● *Multiplex ARMS-PCR (MARMS-PCR)* ● *Mutation* ● *Hematological parameters* ● *HPLC*

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Introduction

β-thalassemia is one of the most common single gene disorders in the world, involving a diverse group of defects in hemoglobin synthesis, all of which result from reduced output of β-globin chains.[1,2] Unlike the α-thalassemias, which are predominantly produced by deletions in the α-globin gene cluster, most β-thalassemias are caused by point mutations, small deletions or insertions within the β-globin gene or its immediate flanking sequences.[2] Over 200 β-thalassemia alleles have been characterized worldwide. Due to the high diversity of mutations in the β-globin gene, mutations in one population will be different from others. However, in each affected ethnic group, a few common mutations together with a variable numbers of rare mutations account for most of the cases.[3-7]

In Thailand, the prevalence of β-thalassemia gene varies from 3 to 9% throughout the country, with more than 30 different mutations of the β-globin genes already identified.[8-11] The Thai population consists of individuals of Thai, Chinese, Malay and Indian ancestry, each of whom has contributed mutations to the pool of known Thai β-thalassemia genes.[5,10,12-18] Amplification refractory mutation system (ARMS) has been widely used for identification of β-thalassemia mutations in various ethno-cultural groups, but it usually detects one mutation per reaction and can be laborious and expensive.[19-21] To overcome this problem, multiplex-PCR protocols using ARMS (MARMS-PCR) have been developed for β-thalassemia detection.[22-23] We now report a non-radioactive MARMS-PCR protocol that is rapid, simple and cost-effective for direct detection of

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19 common β-globin gene mutations found in Thailand. We have investigated hematological indices related to the genotypes and evaluated for coinheritance of α-thalassemia.

Materials and methods

1. Samples

Depending on Hb typing results, a total of 250 peripheral blood samples from individuals diagnosed as β-thalassemia, β-thalassemia/Hb E, or homozygous β-thalassemia, and 100 peripheral blood samples from normal were included in the study. All subjects were more than 1 year of age and attended the routine thalassemia clinic. The study protocol was approved by Committee on Human Rights to Researches Involving Human Subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand (MURA2007/085).

2. Hematological Analysis and Hemoglobin Identification

Hematological indices were analyzed within 2 hours after whole blood collection using an automated Advia 120 blood cell analyzer (Bayer Diagnostic Division, Tarrytown, NY, USA). Blood hemolysate was prepared, and hemoglobin identification was employed using an automated high performance liquid chromatography (Variant HPLC, Bio-Rad Laboratories, California, USA).

3. Primers for MARMS-PCR

Nineteen β-thalassemia mutations commonly found in the Thai population were included in this study. Four sets of allele specific primers were used to differentiate these mutations. Primers, designated as set 1 and set 2 primers, for detection of 8 mutations, including codon 41/42 (-TTCT), codon 17 (A>T), codon 71/72 (+A), IVS I-1 (G>T), IVS I-5 (G>C), nt-28 (A>G), codon 8/9 (+G) and IVS II-654 (C>T) were those described by Bhardwaj U *et al.* 2005 [19] (Table 1). Allele-specific primers developed in this study for detection of the other 11 mutations, namely, codon 14/15 (+G), codon 15 (-T), codon 19 (A>G), codon 26 (G>T), codon 27/28 (+C), codon 35 (C>A), codon 41 (-C), codon 43 (G>T), codon 95 (+A), codon 123-125 (-ACCCCACC), and nt -86 (C>G), were grouped into two primer sets, designated as set 3 and set 4. The grouping was based on prevalence of mutations from previous reports,[10,24,25] location of mutations related to each other by the different amplicon sizes, and the melting temperature of each primer, all of which are important parameters in the development of MARMS-PCR protocol. The internal controls for set 3 and 4 were produced from the forward-3 and reverse-3 primers and from forward-4 and reverse-4 primers respectively, as indicated in Table 1. Genomic sequence of β-globin gene was obtained from GenBank database, accession number NG_000007.

4. MARMS-PCR Assay

Genomic DNA was extracted from 300 μL of the peripheral blood sample using Versagene DNA Purification Kit (Gentra System Inc., USA) according to manufacturer's protocol. MARMS-PCR using set 1 and set 2 primers was according to that of Bhardwaj U *et al.* 2005.[19] MARMS-PCR of the other two primer sets was optimized to obtain the presence of all possible combinations of amplicons. For set 3 and set 4 primers, thermocycling was conducted in a 30 μL volume containing 150 ng of DNA, 200 μM each dNTP, 1.25 mM $MgCl_2$, 0.75 U of GoTaq Flexi DNA polymerase with 1x colorless buffer (Promega, Madison WI, USA), and each primer at the concentration shown in Table 1. Amplification was carried out in a Thermal Cycler 480 (Perkin-Elmer Cetus, USA) as the following sequence: 94 °C for 5 min followed by 30 cycles of 94 °C for 45 sec, 62 °C for 45 sec, and 72 °C for 1 min. Amplicons were separated by electrophoresis in 3% GenePure HiRes agarose gel (ISC Bioexpress, Kaysville, UT) and stained with ethidium bromide and visualized under UV illumination.

5. Multiplex PCR Assay for α-Thalassemia Association

Seven deletional α-thalassemic genes, including α^0-thalassemia (- $-^{SEA}$, - $-^{THAI}$, - $-^{FIL}$, - $-^{MED}$, and $-\alpha^{20.5}$ deletion) and α^+-thalassemia (-$\alpha^{3.7}$ and – $\alpha^{4.2}$ deletion), were detected by single-tube multiplex PCR.[26] Non-deletional α-thalassemic genes encoding Hb Constant Spring (Hb CS) and Hb Pakse (Hb PS) were detected

Table 1 Set 3 and set 4 MARMS-PCR primers

β-thalassemia mutation	Primer sequence	Primer concentration (μM)	Amplicon size (bp)
Set 3 primer			
Forward-3* (F)	TCCAACTCCTAAGCCAGTGC	1.2	
Reverse-3 (R)	CGATCCTGAGACTTCCACACTG	0.2	804
Codon 95 (+A) (R)	GGATCCACGTGCAGCTTTG	0.6	649
Codon 43 (G>T) (R)	TGGACAGATCCCCAAAGGACTA	0.5	497
Codon 35 (C>A) (R)	GAACCTCTGGGTCCAAGGT	1.0	472
Codon 26 (G>T) (R)	ACCTGCCCAGGGCCTA	0.5	310
Codon 19 (A>G) (R)	CACCAACTTCATCCACGCTC	0.4	292
Codon 14/15 (+G) (R)	TTCACCTTGCCCCACCA	0.4	276
Nt -86 (C>G) (R)	GGAGTAGATTGGCCAACCCTAC	1.0	102
Set 4 primer			
Forward-4§ (F)	TCCAACTCCTAAGCCAGTGC	1.0	
Reverse-4 (R)	CGATCCTGAGACTTCCACACTG	0.1	804
Codon 15 (-T) (F)	CTGCCGTTACTGCCCTGG	0.4	558
Codon 27/28 (+C) (F)	TGGTGGTGAGGCCCCT	0.867	516
Codon 41 (-C) (F)	ACCCTTGGACCCAGAGGTTT	1.167	351
Codon 123-125(-ACCCCACC) (F)	TCATGCCTCTTTGCACCATTCT	0.6	316
Codon 123-125(-ACCCCACC) (R)	GCAGCCTGCACTGAATTCTTTG	0.6	

* Forward-3 primer used with the 7 reverse mutation primers (R) to produce each mutant amplicon and with reverse-3 primer to produce internal control amplicon.

§ Reverse-4 primer used with forward mutation primers (F) of codon 15, codon 27/28, and codon 41 to produce each mutant amplicon and with forward-4 primer to produce internal control amplicon.

by MARMS-PCR following the method of Fucharoen et al with some modifications.[27,28]

6. Statistical Analysis

Statistical analysis of quantitative variables was performed using non-parametric One-Sample Kolmogorov-Smirnov test and of homogeneity of variances by using Levene's test. The means of each variable were compared using One-Way ANOVA and independent-samples t-test. P-value of less than 0.05 is considered statistically significant. All statistical calculations were performed using SPSS version 12.

Results

A total number of 250 samples identified by Variant HPLC, including 178 heterozygous β-thalassemia, 2 homozygous β-thalassemia and 70 β-thalassemia/Hb E samples, were investigated by MARMS-PCR and found to contain 252 β-thalassemia alleles. One hundred normal control samples (A_2A with $HbA_2 \leq 3.5\%$ by Variant HPLC) were all negative for β-thalassemia alleles. Figure 1 showed electrophoretic bands of MARMS-PCR amplicons from each primer set. The frequencies of β-thalassemia individuals and of each mutant allele were shown in Table 2 in comparison to the other previous reports.[10,24,25] Amongst the total 252 alleles studied, 107 alleles (42.5%) were codon 41/42 (-TTCT) mutation, the most common mutation found in this study. All mutant alleles in the β-thalassemia samples were identified, except perhaps the allele of a homozygous β-thalassemia sample that was possibly either a compound heterozygozity for codon

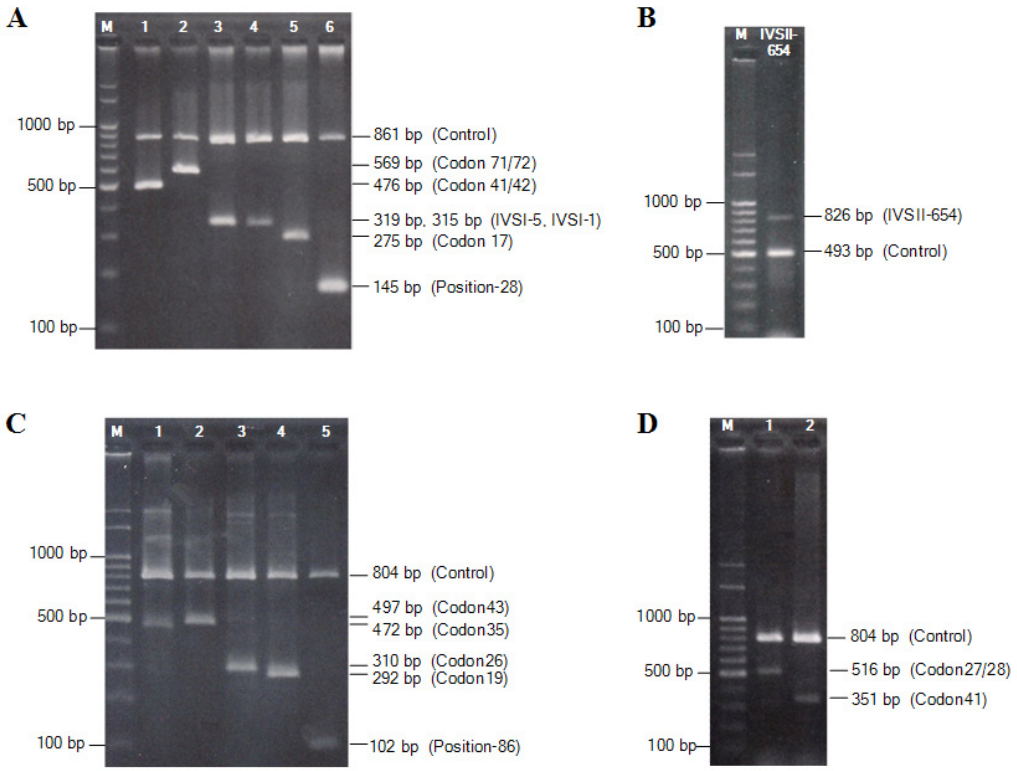

Figure 1. Multiplex ARMS-PCR amplicons using set 1 (**A**), set 2 (**B**), set 3 (**C**) and set 4 (**D**) primers. Each primer set successfully identified β-globin gene mutations as indicated by the presence or absence of the relevant amplicons. For example, lane 1A is codon 41/42 mutation, and lane 2A is codon 71/72 mutation. Internal control was included in each reaction to ensure the effective amplification. Lane M is 100-bp DNA ladder marker (Promega, Madison WI, USA).

41/42 (-TTCT) and an unknown mutation or homozygous codon 41/42 (-TTCT). Five of the 19 β-thalassemia mutations evaluated were not present in this study. Set 1 and set 2 primers allowed identification of 90% of the β-thalassemia samples, and the remaining 7.6% and 2.4% were identified by set 3 and set 4 primers, respectively.

Hemoglobin typing of the 245 β-thalassemia and 100 normal control samples were shown in Table 3. For mild β-thalassemia heterozygotes, such as codon 19 (A>G) and nt -28 (A>G) mutations, Hb A_2 levels (4.9 ± 0.4) of the former mutation were significantly lower than that of severe β-thalassemia mutation, such as IVS II-654 (C>T) ($p < 0.05$), while that of nt -28 (A>G) was surprisingly high (6.0 ± 0.4). All β-thalassemia mutations in heterozygous β-thalassemia samples were associated with high HbA_2 levels (from 5.5 ± 0.2 to 6.3%), and no statistical differences of Hb typing values were found among them, except for the high level of Hb F of codon 27/28 (+C) mutation ($p < 0.05$). Among β-thalassemia/Hb E group, samples with nt -28 (A>G) and codon 19 (A>G) mutations showed higher levels of Hb A than the others; however, their Hb F levels were lower than that of IVS I-5 (G>C) mutation ($p < 0.05$). Both IVS II-654 (C>T) and IVS I-5 (G>C) mutations in β-thalassemia/Hb E showed very low Hb A levels (3.4 ± 2.8 and 5.0 ± 1.7% respectively).

The associations of α-thalassemia among the β-thalassemia samples were shown in Table 4. There was an absence of the presence of α-thalassemia in the majority of β-thalassemia samples (85.2%). There was coinheritance of α^0-thalassemia allele (--^{SEA}/αα) and HbH (--^{SEA}/-$\alpha^{3.7}$) in 2% of heterozygous β-thalassemias. Heterozygous β^0-thalassemia associated with either heterozygous SEA deletion α^0-thalassemia or heterozygous 3.7-kb deletion α^+-thalassemia had higher

Table 2 Frequency of β-thalassemia mutations in Thailand

Mutation	Phenotype	This study n (%)	Winichagoon [25] n (%)	Thein [10] n (%)	Fukumaki [24] n (%)
Codon 41/42 (-TTCT)	β^0	107 (42.5%)	156 (41.6%)	59 (50.9%)	10 (43.6%)
Codon 17 (A>T)	β^0	45 (17.9%)	62 (16.5%)	12 (10.3%)	4 (17.4%)
Nt -28 (A>G)	β^+	28 (11.1%)	34 (9.1%)	12 (10.3%)	1 (4.3%)
IVS II-654 (C>T)	β^+	19 (7.5%)	28 (7.5%)	13 (11.2%)	5 (21.8%)
IVS I-5 (G>C)	β^+	11 (4.4%)	16 (4.3%)	6 (5.2%)	1 (4.3%)
IVS I-1 (G>T)	β^0	8 (3.2%)	5 (1.3%)	2 (1.7%)	1 (4.3%)
Codon 19 (A>G)	β^+	8 (3.2%)	11 (2.9%)	2 (1.7%)	0
Codon 71/72 (+A)	β^0	8 (3.2%)	8 (2.1%)	1 (0.8%)	0
Codon 35 (C>A)	β^0	5 (1.9%)	7 (1.9%)	3 (2.6%)	1 (4.3%)
Codon 27/28 (+C)	β^0	5 (1.9%)	2 (0.5%)	ND	ND
Codon 43 (G>T)	β^0	3 (1.2%)	2 (0.5%)	ND	ND
Codon 26 (G>T)	β^0	2 (0.8%)	0	ND	0
Nt -86 (C>G)	β^+	1 (0.4%)	1 (0.3%)	1 (0.8%)	ND
Codon 41 (-C)	β^0	1 (0.4%)	2 (0.5%)	ND	0
Codon 95 (+A)	β^0	0	1 (0.3%)	ND	ND
Codon 8/9 (+G)	β^0	0	0	ND	0
Codon 14/15 (+G)	β^0	0	1 (0.3%)	1 (0.8%)	ND
Codon 15 (-T)	β^0	0	1 (0.3%)	ND	ND
Codon 123-125 (-ACCCCACC)	β^0	0	ND	ND	0
Codon 26 (Hb E)	-	ND	2 (0.5%)	ND	ND
619 bp deletion	β^0	ND	5 (1.3%)	ND	ND
3.4 kb deletion	β^0	ND	1 (0.3%)	ND	ND
105 bp deletion	β^0	ND	0	ND	ND
Uncharacterized	-	1 (0.4%)	30 (8.0%)	4 (3.4%)	ND
Total		**252**	**375**	**116**	**23**

ND = not done

MCV and higher Hb levels compared with those having normal α-globin genotype (Table 5). Heterozygous β^0-thalassemia coexisting with heterozygous α^0-thalassemia had no difference in HbA_2 levels from those having normal α-globin genotype (Table 5). β^0-thalassemia/Hb E associated with heterozygous 3.7-kb deletion α^+-thalassemia showed lower MCV compared with those with normal α-globin genotype. On the other hand, the percentage of Hb E was higher, while the percentage of Hb F was lower. Coinheritance of α-thalassemia had no significant effect on hematological parameters of heterozygous β^+-thalassemia and β^+-thalassemia/Hb E (Table 5).

Discussion

In this study, we used 4 sets of primers in MARMS-PCR technique to detect the 19 β-thalassemia mutations commonly found in Thai individuals, 2 sets of which (set 1 and set 2) were those previously reported,[19] while the other 2 sets were developed particularly for the study. Only 14 out of 19 β-thalassemia mutations were identified, and the remaining 5 mutations were probably too infrequent to be seen among the limited sample size (n = 250). Set 1 and set 2 primers were able to identify mutations in only 90% of β-thalassemia samples. The

Table 3 Hemoglobin (Hb) typing associated with β-thalassemia mutations

		Hb typing values (mean ± SD)		
		%Hb F	%Hb A_2/Hb E	%Hb A
Heterozygous β-thalassemia (n = 178)				
β^+	Codon 19 (n = 6)	1.6 ± 1.7	4.9 ± 0.4	84.3 ± 2.3
	Nt -28 (n = 20)	1.6 ± 1.1	6.0 ± 0.4	82.8 ± 1.2
	IVS II-654 (n = 17)	1.8 ± 1.8	5.6 ± 0.3	83.4 ± 1.2
	IVS I-5 (n = 8)	1.5 ± 1.1	5.3 ± 0.3	83.3 ± 1.7
β^0	Codon 41/42 (n = 77)	1.5 ± 1.8	6.0 ± 0.6	82.7 ± 2.5
	Codon 17 (n = 27)	1.3 ± 1.1	5.9 ± 0.5	82.9 ± 1.8
	IVS I-1 (n = 7)	2.3 ± 1.7	5.6 ± 0.5	82.0 ± 2.2
	Codon 71/72 (n = 5)	1.7 ± 1.5	5.9 ± 0.9	82.1 ± 2.1
	Codon 35 (n = 3)	2.2 ± 0.4	6.2 ± 0.2	82.2 ± 1.6
	Codon 27/28 (n = 5)	5.6 ± 3.1	5.5 ± 0.2	80.4 ± 2.5
	Codon 43 (n = 2)	2.8 ± 1.3	5.8 ± 0.1	82.6 ± 2.2
	Codon 26 (n = 1)	0.8	6.3	84.1
β-Thalassemia/Hb E (n = 64)				
β^+	Codon 19 (n = 2)	5.1 ± 0.6	59.7 ± 1.2	30.6 ± 0.4
	Nt -28 (n = 7)	17.5 ± 5.7	59.1 ± 6.7	20.3 ± 0.9
	Nt -86 (n = 1)	11.6	63.0	27.3
	IVS II-654 (n = 2)	34.4 ± 8.6	64.4 ± 4.2	3.4 ± 2.8
	IVS I-5 (n = 3)	36.0 ± 18.1	60.2 ± 13.3	5.0 ± 1.7
β^0	Codon 41/42 (n = 28)	37.6 ± 14.3	60.1 ± 14.3	-
	Codon 17 (n = 16)	37.2 ± 15.0	61.9 ± 14.2	-
	Codon 71/72 (n = 2)	33.9 ± 8.6	64.3 ± 10.3	-
	Codon 35 (n = 1)	47.5	46.9	-
	Codon 43 (n = 1)	41.5	49.2	-
	Codon 26 (n = 1)	34.9	67.1	-
Homozygous β-thalassemia (n = 2)				
	Codon 41/42 (n = 2)[§]	84.8 ± 1.4	1.7 ± 2.1	-
Normal (n = 100)		1.0 ± 1.3	3.3 ± 0.5	86.1 ± 2.2

* Six samples of β-thalassemia contaminated with transfused blood were not included in this table.

§ One sample was compound heterozygous of codon 41/42 and nt -28; the other was homozygous codon 41/42 or possible compound heterozygous of codon 41/42 and an unknown mutation

Table 4 Frequency of β-thalassemia in association with α-thalassemia

α-globin genotype	Heterozygous β-thalassemia	β-thalassemia/Hb E	Homozygous β-thalassemia	Total
αα/αα	151 (60%)	60 (24%)	2 (1%)	213 (85%)
$-\alpha^{3.7}/\alpha\alpha$	17 (7%)	8 (3%)	0	25 (10%)
$-\alpha^{4.2}/\alpha\alpha$	1 (0.5%)	0	0	1 (0.5%)
$\alpha^{CS}\alpha/\alpha\alpha$	3 (1%)	2 (1%)	0	5 (2%)
$-\alpha^{3.7}/\alpha^{CS}\alpha$	1 (0.5%)	0	0	1 (0.5%)
$--^{SEA}/\alpha\alpha$	4 (1%)	0	0	4 (1%)
$--^{SEA}/-\alpha^{3.7}$	1 (0.5%)	0	0	1 (0.5%)
Total	**178 (71%)**	**70 (28%)**	**2 (1%)**	**250 (100%)**

Table 5 Hematological data of β-thalassemia coexistence with α-thalassemia

Hematological parameter	No α-thal	α-thal 2 trait	α-thal 1 trait	Hb H disease
β^+-thal trait	(n = 47)	(n = 3)		
Hb	11.9 ± 1.4	12.9 ± 1.8		
MCV	65.9 ± 6.5	70.5 ± 6.7		
%Hb A_2	5.6 ± 0.5	5.8 ± 0.9		
β^0-thal trait	(n = 104)	(n = 14)	(n = 4)	(n = 1)
Hb	10.7 ± 1.6	11.3 ± 1.8	12.4 ± 1.8	9.8
MCV	60.9 ± 5.3	65.3 ± 4.7*	67.7 ± 3.1*	56.2
%Hb A_2	5.9 ± 0.5	6.3 ± 0.5*	5.8 ± 0.9	4.8
β^+-thal/Hb E	(n = 13)	(n = 2)		
Hb	9.0 ± 1.7	8.9 ± 2.7		
MCV	58.4 ± 7.2	61.7 ± 0.3		
%Hb F	21.8 ± 14.4	18.7 ± 11.5		
%Hb A_2/E	59.3 ± 7.0	67.3 ± 0.3		
β^0-thal/Hb E	(n = 41)	(n = 6)		
Hb	7.6 ± 1.4	8.6 ± 0.5		
MCV	62.2 ± 9.0	49.8 ± 4.2*		
%Hb F	40.8 ± 12.2	19.2 ± 8.8*		
%Hb A_2/E	57.0 ± 12.0	78.8 ± 6.9*		

*p-value < 0.05

five most common mutations found in this study were in agreement with those of Winichagoon *et al*,[25] but differed from those reported by Fukumaki *et* al,[24] and Thein *et al*.[10] Differences may be due to the larger sample sizes used in this study and that of Winichagoon *et* al.[25] Nevertheless, the most prevalent mutation identified in all studies is codon 41/42 (-TTCT).

Heterozygous β-thalassemia associated with the inheritance of a single β-thalassemia allele, either β^0 or β^+, is characterized by a mild anemia with hypochromic microcytic red blood cells, elevated levels of Hb A_2 and variable levels of Hb F.[29,30] Codon 19 (A>G) mutation had the lowest level of HbA_2 (4.9 ± 0.4%) among all heterozygous β-thalassemia mutations in this study. A similar observation has been previously reported in two patients with heterozygosity for codon 19 (A>G) mutation manifesting a mild hypochromic microcytosis without anemia and Hb A_2 levels in the range of 3.4 to 3.8%[33]. The nt-28 (A>G) transcriptional mutation, mild β^+-thalassemia mutation, showed higher HbA_2 level than other β^+-thalassemia mutations. *In vitro* transient expression assays have indicated that these defects allow β-globin mRNA output ranging from 20% to 30% of normal,[32,33] enough to contribute to the relatively mild phenotype of these β^+-thalassemias.[34] In contrast, the IVS II-654 (C>T) mutation results in the formation of an abnormally spliced mRNA, but does not abolish normal RNA processing entirely so that a significant amount (over 15%) of normally processed β-globin mRNA is produced, resulting in severe β^+-thalassemia.[35]

Compound heterozygosity for Hb E and β-thalassemia results in variable severity, mostly moderate.[1,30,36] Patients with compound heterozygosity of Hb E and mild β^+-thalassemia, such codon 19 (A>G) and nt -28 (A>G) mutations, usually have a mild thalassemic phenotype. Compound heterozygosity of severe β^+-thalassemia, such as IVS II-654 and IVS I-5, leads to severe hemolytic stress with Hb F levels increasing to that of β^0-thalassemia/HbE disease[36]. The lowest Hb F (< 10%) and the highest Hb A levels were found with codon 19 (A>G) mutation. Yang *et al*[37] have reported two patients with β^{cd19}/β^E thalassemia with low Hb F levels of 3.9% and 9.7%.

Codon 19 (A>G) mutation results in the synthesis of a hemoglobin variant, Hb Malay (β19: Asn>Ser), which co-elutes with Hb A in HPLC-hemoglobin typing, and thus the putative Hb A observed in these patients is Hb Malay.[38] The severe phenotype seen in IVS II-654 (C>T) or IVS I-5 (G>C) mutation with Hb E can be attributed to the low levels of normal β-globin mRNA produced.[34] Laosombat *et* al[39] and Yang *et al*[37] have reported patients with compound heterozygosity for IVS I-5 (G>C) and Hb E, in which the amounts of Hb A range from 5.5 to 7.7% and 2.7 to 5.8% respectively.

In Thailand, various forms of α-thalassemia are common, with the prevalence of 3-4% for α^0-thalassemia, 20-30% for α^+-thalassemia and at least 4% for Hb Constant Spring (Hb CS) genes.[11] Consequently, coinheritance of α-thalassemia with β-thalassemia is anticipated. In our study, 13% of β-thalassemia samples were associated with either α^0- or α^+-thalassemia genes, of which 3.7-kb deletion α^+-thalassemia was the most prevalent. Concomitant inheritance of α-thalassemia has been suggested to produce a more balanced α-globin and β-globin chain synthesis ratio and thereby generates mildness of anemia in β-thalassemia disease. From our study, one case with coexisting Hb H disease in heterozygous β^0-thalassemia showed HbA_2 in the level of diagnostic criteria of heterozygous β-thalassemia. However, α-thalassemia is a factor affected HbA_2 level and can cause a pitfall in diagnosis of heterozygous β-thalassemia. Our studies showed that coinheritance of SEA-deletion α^0-thalassemia with heterozygous β^0-thalassemia produces higher MCV and Hb levels than coinheritance of 3.7-kb deletion α^+-thalassemia or normal α-globin gene. Xu *et al*[40] reported that MCV and α- to β-globin ratio values were raised to varying degrees in co-inherited α- and β-thalassemia carriers. In contrast, decreased MCV and MCH in coinheritance of β^0-thalassemia/Hb E with 3.7-kb deletion α^+-thalassemia are presumed to be related to a decrease in Hb F and an increase in Hb E levels, consistent with the notion that Hb F level is decreased when a deletional α-thalassemia mutation coexists with other β-globin gene mutations.[41]

In summary, we have reported a successful development of multiplex (M)ARMS-PCR to identify a number of common and rare Thai β-thalassemia mutations without amplifying nonspecific sequences. This MARMS-PCR assay is robust, accurate, cost-effective and labor-efficient, and should find a place in the routine detection of mutations of β-globin gene causing β-thalassemia in the Thai population.

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การตรวจวินิจฉัยเบต้าธาลัสซีเมียมิวเตชั่น 19 ชนิด ด้วยเทคนิคมัลติเพล็กซ์อาร์มสพีซีอาร์

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บทคัดย่อ : *เบต้าธาลัสซีเมียเป็นโรคทางพันธุกรรมที่มีความผิดปกติของเม็ดเลือดแดง ซึ่งมีชนิดของความผิดปกติหลากหลายมาก ในไทยพบความถี่ของเบต้าธาลัสซีเมียประมาณร้อยละ 3-9 การศึกษาครั้งนี้ได้ทำการตรวจวินิจฉัยเบต้าธาลัสซีเมีย 19 ชนิด ด้วยวิธีมัลติเพล็กซ์อาร์มสพีซีอาร์ โดยแบ่งเป็น 4 ชุดปฏิกิริยา จากตัวอย่างทั้งสิ้น 250 ตัวอย่าง พบว่าเป็นพาหะของเบต้าธาลัสซีเมีย 178 ตัวอย่าง เป็นโฮโมซัยกัสเบต้าธาลัสซีเมีย 2 ตัวอย่าง และเป็นโรคเบต้าธาลัสซีเมียร่วมกับฮีโมโกลบินอี 70 ตัวอย่าง ทั้งนี้จากชุดตรวจสำหรับมิวเตชั่น 19 ชนิด ตรวจพบได้ 14 ชนิด ซึ่งร้อยละ 83 เป็นชนิดที่พบบ่อย 5 อันดับแรกในคนไทย คือ codon 41/42 (-TTCT), codon 17 (A>T), nt-28 (A>G), IVS II-654 (C>T) และ IVS I-5 (G>C) การพบอัลฟ่าธาลัสซีเมียในรายที่เป็นพาหะของเบต้าธาลัสซีเมียจะมีความสมดุลระหว่างอัลฟ่าและเบต้าโกลบินกว่า โดยมีค่าฮีโมโกลบิน MCV และ MCH สูงขึ้น ขณะที่การพบอัลฟ่าธาลัสซีเมียชนิดยีนแหว่งขนาด 3.7 กิโลเบสในรายที่เป็นโรคเบต้าธาลัสซีเมียร่วมกับฮีโมโกลบินอี มีค่า MCV และ MCH ลดลง ซึ่งคาดว่าน่าจะสัมพันธ์กับค่าฮีโมโกลบินเอฟที่ลดลงและค่าฮีโมโกลบินอีที่สูงขึ้น*

Key Words : ● *ธาลัสซีเมีย* ● *มัลติเพล็กซ์อาร์มสพีซีอาร์* ● *มิวเตชั่น* ● *ค่าการตรวจทางโลหิตวิทยา* ● *เครื่องวิเคราะห์ปริมาณฮีโมโกลบินอัตโนมัติ HPLC*

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