Prevalence and Hematological Parameters of Thalassemia in Tha Kradarn Subdistrict Chachoengsao Province, Thailand

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Objective: *To determine the prevalence, molecular characteristics and hematological study of thalassemia in Tha Kradarn Subdistrict Chachoengsao Province.*
Material and Method: *The present study population consisted of 266 participants from Moo 19 Baan Na-Ngam, Chachoengsao Province, Thailand. After blood collection, all samples were screened for thalassemia by initial screening with the OF and DCIP tests and additional testing by CBC, RBC indices, hemoglobin typing and determination of Hb A_2 and Hb E. All common α-thalassemia mutations were determined using the PCR with allele specific primers and Gap PCR for common deletions.*
Results: *The prevalence of α-thal 1, α-thal 2 and β-thal were found as 2.72%, 11.26% and 0.97%, respectively. Regarding the abnormal hemoglobins, the prevalence of Hb E, Hb Constant Spring and Hb Pakse was 38.45%, 3.69% and 0.78%, respectively. MCV and MCH were significantly different between β-thalassemia as well as α-thal 1 carriers and normal subjects. In all α-thal 1 traits, it was found that the MCV and MCH were less than 75 fL and 25 pg, therefore, these parameters can be used for α-thal 1 screening.*
Conclusion: *In the present study, the prevalence of thalassemia was similar to previous studies. Moreover, using the combination of OF and DCIP tests compared with MCV, MCH and DCIP tests for the initial thalassemia screening, it was found that the OF and DCIP tests gave more false positive results, which increased the need for further Hb typing. Hence, the MCV and MCH combined with DCIP tests provide cost minimization and practical for a large population-based screening program.*

Keywords: *Thalassemia, OF test, DCIP test, RBC indices, Hb typing, PCR for α-thalassemia*

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Thalassemia and hemoglobinopathy are the most common genetic disorders in Thailand and Southeast Asian countries[1-3]. Mutations of globin genes result in reduced or absent production of the globin chain. Thalassemia is mainly divided into α- and β-thalassemias. Most α-thalassemia is caused by gene deletions and clinical severity depends on the number of α-genes deleted. The most common single α-gene deletion is 3.7 kb deletion ($-\alpha^{3.7}$) and 4.2 kb deletion ($-\alpha^{4.2}$) is rare. Both a-gene deletions are SEA-type ($-^{SEA}$) for which approximately a 20 kb gene cluster is deleted and THAI type ($-^{THAI}$) for which a 34-38 kb α-gene cluster is deleted. On the other hand, β-thal is usually due to a point mutation. Its clinical severity

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depends on the number of β-globin chain productions. In Thailand, HbE, Hb Constant Spring (HbCS) and HbPakse (HbPS) can be found. The Hb E results from a base substitution at the codon 26 of β-gene, whereas HbC S and Hb PS result from alterations at the terminal codon of α_2-gene that changes to CAA and TAT, respectively[4,5]. In Thailand, the prime targets of prevention and control of severe thalassemia are homozygous α-thal 1 (Hb Bart's hydrop fetalis syndrome), homozygous β-thalassemia and β-thalassemia and Hb E disease. Prevalence data is important for prevention and control strategies and the prevalence of thalassemia in different parts of Thailand has been reported[5-8]. Routinely, different levels of screening tests for thalassemia have been introduced such as the red cell one-tube osmotic fragility test (OF) combined with the dichlorophenol-indolphenol (DCIP) precipitation test; complete blood count (CBC) and red blood cell (RBC) indices, especially mean corpuscular volume (MCV) and red cell distribution width (RDW)[9-18]. However, additional confirmatory tests such as hemoglobin typing for the determination of Hb A_2 and Hb E and DNA analysis are needed[19].

A recent study among 266 volunteers, living in Baan Na-Ngam, Chachoengsao Province, Thailand showed that the prevalence of β-thal traits was 1.1%, Hb E trait was 35.3% and homozygous Hb E was 5.3%. Since DNA analysis was not performed, α-thal 1 and α-thal 2 traits could not be excluded[20]. Therefore, the purpose of the present study was to facilitate the effectiveness of diagnostic screening of thalassemias and hemoglobinopathies in this remote rural area of Thailand.

Material and Method

Samples

Altogether, 266 subjects were recruited from Moo 19, Baan Na-Ngam, Chachoengsao Province, Thailand. Three milliliter of peripheral EDTA blood samples was obtained from each subject. The subjects were 105 males and 161 females, with ages ranging from 7 to 49 with a mean age of 35.4 years. Informed consent was obtained from all subjects. After collection, all samples were immediately screened for thalassemia using the modified one-tube OF test. They were screened for Hb E using the modified DCIP precipitation test. All blood samples were stored in a cool container and transferred within six hours to the Department of Pediatrics, Phramongkutklao Hospital for determination of CBC and erythrocyte indices using the Coulter ONYX automated blood cell counter (Coulter Electronics, Hialeah, FL, USA).

The present research was approved by medical committee of institution.

Screening test

The OF test was performed as described by Fucharoen G and Nathalang O[15,21]. A 0.34% buffer saline solution was prepared. Then a sample of 20 μL of whole blood was mixed with 2 mL of the saline solution in a 13 mm x 100 mm test tube and left at room temperature for 15 minutes before being interpreted. The modified DCIP test kit, using a clear reagent, was used in of the present study[15,21]. For the DCIP precipitation test, 20 μL of whole blood was added to 2 mL of a modified DCIP reagent and then incubated at 37°C for 15 minutes before adding 20 μL of stopping agent. Both tests were interpreted as negative or positive by visualization. Negative samples are clear, and positive samples are cloudy. For of the present study, suspicious samples with very little cloudiness were considered to be positive.

Hemoglobin typing

Hemoglobin typing was conducted by automated low-performance liquid chromatography; LPLC (Hb GOLD, Drew, UK)[22].

DNA analysis

DNA extraction was performed in all 266 blood samples using JETQUICK blood & cell culture DNA spin kits (GENOMED GmbH, Lohne, Germany). All common α-thalassemia mutations, including α-thal 1 (SEA type), α-thal 2 (3.7- and 4.2-kilobase deletions), Hb CS and Hb PS were determined using PCR method with allele specific primers described previously[23-26]. Briefly, the reaction mixture for PCR in 20 mL contained 2 mL of DNA (100-300 ng/mL), 0.5 mL of 10 pmol of each primer, 2.2 mL (200 mmol/mL) of each dedoxynucleoside triphosphate and 0.12 unit of Taq Polymerase (Promega, WI, USA). The reaction mixture was placed in a PTC-200 Thermal Cycler (MJ Research Inc., MA, USA) and Px2 Thermal Cycler (Thermo Electron Corporation, MA, USA). The PCR program for α-thal 1, Hb CS and Hb PS is an initial denaturation step at 94°C for 3 minutes, followed by 30 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 40 seconds with a final extension at 72°C for 5 minutes. The PCR program for α-thal2 is an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of 94°C for 1 minute, 55°C for 40 seconds, 72°C for 40

seconds with a final extension at 72°C for 7 minutes. The PCR products were electrophoresed in 2% agarose gel in 1X TBE buffer and the bands were visualized using a UV transilluminator. The PCR products of positive samples for α-thal 1 showed 314 bp and 195 bp, while negative samples showed only 314 bp. The PCR products of positive samples for HbCS, produced 578 bp and 190 bp whereas negative samples produced only 578 bp. The positive PCR products for heterozygous Hb PS showed 253 bp and 180 bp with the 391 bp as an internal control, while the positive PCR products of homozygous PS showed only 253 bp and 391 bp and the negative samples produced only 391 bp. The PCR products of positive samples for α-thal 2 (3.7 del) showed 1,800 bp and 578 bp while negative samples showed only 578 bp. Regarding α-thal2 (4.2 del), the PCR products of positive samples showed 1,761 bp and 227 bp while negative samples showed only 227 bp.

Statistical analysis

Statistical analysis was performed by Statistical Package for Social Science (SPSS) for Windows release 11.1 (SPSS Inc., Chicago, Illinois, USA). Means and standard deviations (SD) of various numerical parameters were calculated. The Mann-Whitney U test was used to compare the mean Hb, Hct, MCV, MCH, MCHC and RDW levels among the groups. A p-value of less than 0.05 was considered as significant.

Results

The prevalence of a-thalassemias in Baan Na-Ngam, Chachoengsao Province is shown in Table 1. All 266 subjects were divided into four groups depending on the results of the OF and DCIP tests: -/-, -/+, +/- and +/+. It was found that of 154 subjects with normal hemoglobin typing results (A_2A), 8 had a-thal1 trait (SEA type), 19 had α-thal 2 trait ($-\alpha^{3.7}/\alpha\alpha$), 2 had homozygous α-thal 2 13 had Hb CS trait, 3 had Hb PS trait, 2 had a combination of α-thal 2 and Hb CS trait and 1 had a combination of α-thal 2 and Hb PS trait. Importantly, all of 8 α-thal 1 traits (SEA type) were positive for the OF test. Of three α-thalassemia traits with +/- pattern, 1 had a combination with α-thal1 trait and 1 had a combination of α-thal2 trait. Of 94 Hb E traits with -/+ and +/+ patterns, 5 had α-thal 1 trait, 10 had α-thal 2 trait, 2 had homozygous α-thal 2 8 had Hb CS trait, 3 had Hb PS trait, 1 had a combination of α-thal 2 and Hb CS trait. Of 14 homozygous Hb E with +/+ pattern, 3 had α-thal 2 trait, 1 had homozygous α-thal 2, 1 had Hb CS trait, 1 had homozygous Hb CS and 1 had a combination of α-thal 1 and Hb PS trait. Additionally, the 4.2 kb deletion ($-\alpha^{4.2}$) was not found in of the present study. Furthermore, 1 sample of αα/αα genotype showed an abnormal Hb pattern, which could not be determined by automated LPLC. This sample was further tested by DNA sequencing and identified as Hb Hope trait.

The hematological laboratory findings among various groups of subjects are shown in Tables 2 and 3. It was found that the RBC indices, including MCV, MCH and MCHC of the α-thal 1 and -thal 2 traits, Hb CS traits, homozygous Hb E, Hb E traits, Hb E traits with α-thal 1 and Hb E traits with α-thal 2 were significantly lower than normal subjects ($p < 0.05$). In addition, the MCV, MCH and the percentage of Hb E for Hb E traits with α-thal 1 traits were also significantly lower than Hb E traits ($p < 0.05$). Interestingly, even though the percentage of Hb E for Hb E traits with α-thal 2 traits and Hb E traits with Hb CS traits were lower than Hb E traits ($p < 0.05$), the MCV and MCH were slightly higher than Hb E traits ($p < 0.05$).

The sensitivity and specificity of a combination of OF and DCIP tests to screen thalassemia and hemoglobinopathies in 266 subjects were 82.5% and 92.5%. On the other hand, the sensitivity and specificity of a combination of MCV and DCIP tests were 83.8% and 94.3%, as shown in Table 4.

Discussion

The aim of the prevention and control program for thalassemia in Thailand and other Southeast Asian countries is to prevent birth of new cases with three severe thalassemic diseases including homozygous α-thal 1 causing Hb Bart's hydrops fetalis, homozygous β-thalassemia and β-thalassemia/Hb E disease[27]. The OF and DCIP tests are routinely used as a screening protocol for thalassemia and hemoglobinopathies. However, the use of CBC and RBC indices obtained by automated blood cell analyzer have been added[17,28,29]. Individuals with low MCV (< 80 fL) and MCH (< 27 pg) usually have further investigation by electrophoresis or HPLC or LPLC or DNA analyses in order to identify α-thalassemias, β-thalassemia and Hb E carriers[30].

A previous study revealed that using the OF and DCIP tests as initial screening tests combined with additional tests such as CBC and hemoglobin typing, the prevalence of β-thal traits was 1.1%, Hb E traits, 35.3% and homozygous Hb E, 5.3%[20]. In the present study, additional DNA analyses for α-thalassemias, Hb CS and Hb PS were performed. Concerning 154 subjects

Table 1. Prevalence of α-thalassemias in 266 subjects

HbType(OF/DCIP)	α-thalassemia genotype										
	n	αα/αα	$-\alpha^{3.7}/\alpha\alpha$	$-\alpha^{3.7}/-\alpha^{3.7}$	$-^{SEA}/\alpha\alpha$	$\alpha^{cs}\alpha/\alpha\alpha$	$\alpha^{cs}\alpha/\alpha\,^{cs}\alpha$	$-\alpha^{3.7}/\alpha^{cs}\alpha$	$\alpha^{PS}\alpha/\alpha\alpha$	$-\alpha^{3.7}/\alpha\,^{PS}\alpha$	$-/\alpha\,^{PS}\alpha$
Normal β^A/β^A	154										
(-/-)	126	98	15	0	0	9	0	1	3	0	0
(-/+)	3	2	0	0	0	1	0	0	0	0	0
(+/-)	23	6	4	2	7	2	0	1	0	1	0
(+/+)	2	0	0	0	1	1	0	0	0	0	0
β-thal trait β^+/β^A	3										
(-/-)	0	0	0	0	0	0	0	0	0	0	0
(-/+)	0	0	0	0	0	0	0	0	0	0	0
(+/-)	3	1	1	0	1	0	0	0	0	0	0
(+/+)	0	0	0	0	0	0	0	0	0	0	0
Hb E trait β^A/β^E	94										
(-/-)	0	0	0	0	0	0	0	0	0	0	0
(-/+)	57	45	3	0	0	6	0	0	3	0	0
(+/-)	0	0	0	0	0	0	0	0	0	0	0
(+/+)	37	20	7	2	5	2	0	1	0	0	0
Homo E β^E/β^E	14										
(-/-)	0	0	0	0	0	0	0	0	0	0	0
(-/+)	0	0	0	0	0	0	0	0	0	0	0
(+/-)	0	0	0	0	0	0	0	0	0	0	0
(+/+)	14	7	3	1	0	1	1	0	0	0	1
Hb Hope trait β^A/β^{Hope}	1										
(-/-)	1	1	0	0	0	0	0	0	0	0	0

Table 2. RBC indices and Hb A_2 in normal, α-thalassemias, Hb CS and Hb PS in various combinations

Hb type	Genotype	n (%)	Hb A_2 (%)	RBC (x10^{12}/L)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)
A_2A	Normal (αα/αα)	106 (68.8)	2.54 ± 0.35	4.52 ± 0.44	13.60 ± 1.42	40.26 ± 3.90	89.18 ± 5.55	30.13 ± 2.18	33.77 ± 0.86	13.40 ± 1.20
A_2A	α-$thal_1$ trait (--/αα)	8 (5.2)	2.25 ± 0.27	5.60 ± 0.49*	12.24 ± 1.04*	38.18 ± 3.23	68.16 ± 2.48*	21.94 ± 0.72*	32.20 ± 0.71*	16.14 ± 2.96*
A_2A	α-$thal_2$ trait (-$\alpha^{3.7}$/αα)	19 (12.3)	2.49 ± 0.37	4.75 ± 0.47*	13.27 ± 1.29	39.96 ± 3.70	84.23 ± 4.64*	27.98 ± 1.64*	33.21 ± 0.71*	13.62 ± 0.85
A_2A	Homozygous α-$thal_2$ (-$\alpha^{3.7}$/-$\alpha^{3.7}$)	2 (1.3)	2.45 ± 0.21	5.64 ± 0.40	12.80 ± 1.41	39.50 ± 4.67	70.00 ± 3.25	22.70 ± 0.85	32.40 ± 0.28	15.40 ± 0.85
A_2A	Hb CS trait (α^{CS}α/αα)	13 (8.4)	2.16 ± 0.41	4.68 ± 0.50	12.82 ± 1.49	38.75 ± 4.26	83.08 ± 7.52*	27.47 ± 2.68*	33.03 ± 0.72*	14.48 ± 2.36
A_2A	α-$thal_2$ trait with Hb CS (-$\alpha^{3.7}$/α^{CS}α)	2 (1.3)	2.10 ± 0.28	5.21 ± 0.45	12.10 ± 0.28	38.70 ± 1.56	74.40 ± 3.54	23.30 ± 1.41	31.30 ± 0.42	15.15 ± 2.62
A_2A	Hb PS trait (α^{PS}α/α)	3 (2.0)	1.97 ± 0.4	5.02 ± 0.57	13.23 ± 1.82	40.43 ± 4.95	80.50 ± 3.20	26.27 ± 1.07	32.67 ± 0.71	13.93 ± 0.49
A_2A	α-$thal_2$ trait with Hb PS (-$\alpha^{3.7}$/α^{PS}αα)	1 (0.7)	1.9	5.25	12.5	39.1	74.4	23.8	31.9	13.5

n = Number tested, * p-value < 0.05

Table 3. RBC indices and HbE in homozygous HbE, HbE trait in various combinations with α-thalassemias, Hb CS and Hb PS

Hb type	Genotype	n (%)	Hb E (%)	RBC (x10^{12}/L)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)
EA	Hb E trait(αα/αα)	65 (69.2)	29.4 ± 1.78	4.91 ± 0.54	12.81 ± 1.42	38.61 ± 4.05	78.81 ± 4.06	26.14 ± 1.58	33.16 ± 0.78	14.05 ± 0.94
EA	α-$thal_1$ trait (--/αα)	5 (5.3)	21.4 ± 0.56*	5.42 ± 0.57	12.42 ± 1.21	37.84 ± 3.61	69.96 ± 1.44*	22.94 ± 0.64*	32.82 ± 0.94	14.74 ± 0.98
EA	α-$thal_2$ trait (-$\alpha^{3.7}$/αα)	10 (10.6)	28.12 ± 2.59*	4.78 ± 0.39	13.36 ± 1.41	40.47 ± 3.77	84.69 ± 5.34*	27.95 ± 2.24*	32.97 ± 0.79	14.04 ± 1.67
EA	Homozygous α-$thal_2$ (-$\alpha^{3.7}$/-$\alpha^{3.7}$)	2 (2.1)	21.7 ± 0.14*	4.90 ± 0.14	12.10 ± 0.28	37.35 ± 0.78	76.25 ± 3.75	24.75 ± 1.20	32.45 ± 0.07	13.95 ± 0.49
EA	Hb CS trait (α^{CS}α/αα)	8 (8.5)	27.76 ± 1.37*	4.67 ± 0.43	13.25 ± 0.98	39.98 ± 3.16	85.80 ± 5.09*	28.49 ± 2.11*	33.18 ± 0.78	13.45 ± 0.75
EA	α-$thal_2$ trait with Hb CS (-$\alpha^{3.7}$/α^{CS}α)	1 (1.1)	21.3	4.53	11.7	36.5	80.5	25.9	32.2	16.0
EA	Hb PS trait (α^{PS}α/αα)	3 (3.2)	27.33 ± 2.32	5.15 ± 0.54	13.47 ± 1.63	40.90 ± 4.46	79.47 ± 3.23	26.20 ± 1.25	32.93 ± 0.46	14.10 ± 0.36
Total		94 (100)								
EE	Homozygous E(αα/αα)	7	87.64 ± 4.77*	5.71 ± 0.39*	12.04 ± 0.85	37.49 ± 2.23	65.81 ± 4.51*	21.13 ± 1.56*	32.13 ± 0.78*	15.66 ± 0.94*
EE	α-$thal_2$ trait (-$\alpha^{3.7}$/αα)	3	88.63 ± 0.5	5.42 ± 0.41	11.50 ± 1.51	35.70 ± 3.70	65.77 ± 3.31	21.17 ± 1.36	32.17 ± 0.93	15.43 ± 0.85
EE	Hb CS trait (α^{CS}α/αα)	1	83.4	5.27	11.4	35.9	68.2	21.7	31.8	15.1
EE	Homozygous α-$thal_2$ (-$\alpha^{3.7}$/-$\alpha^{3.7}$)	1	90.2	5.55	8.7	28.7	51.8	15.6	30.2	39.2
EE	Homozygous Hb CS (α^{CS}α/α^{CS}α)	1	90.3	4.81	11.4	35.5	73.8	23.6	32.0	13.3
EE	α-$thal_1$ trait with Hb PS (-/α^{PS}α)	1	85.0	5.19	11.4	36.0	69.4	22.0	31.7	14.4
Total		14 (100)								

n = Number tested, * p value < 0.05

Table 4. Comparison of screening tests with Hb typing and PCR for α-thalassemias

Screening tests	Hb typing & PCR for α-thalassemias		Total
	Positive	Negative	
A) OF & DCIP			
Positive	132	8	140
Negative	28	98	126
Total	160	106	266
B) MCV & DCIP			
Positive	134	6	140
Negative	26	100	126
Total	160	106	266

A) Sensitivity = 82.5%, specificity = 92.5%, positive predictive value = 94.3% and negative predictive value = 77.8%
B) Sensitivity = 83.8%, specificity = 94.3%, positive predictive value = 95.7% and negative predictive value = 79.4%

with normal hemoglobin typing results, 48 (31.2%) were positive for α-thalassemias, Hb CS and Hb PS. The most commonly found were α-thal2 traits (12.3%), Hb CS traits (8.4%), α-thal 1 traits (5.2%) and Hb PS traits (2.0%). Only α-thal 2 ($-\alpha^{3.7}$) was found in this population, similar to previous findings in the Thai population[31]. Regarding the OF test, 0.34% buffer saline solution was used instead of 0.36% OF in order to minimize false positive results as recommended in previous studies[21,30]. Additionally, in of the present study, the MCV and MCH were significantly reduced in almost all thalassemia genotypes. Therefore, they are recommended as primary screening indicators for further diagnosis by specific DNA analysis of α-thal1, especially when MCV and MCH are less than 75 fL and 25 pg, respectively. Moreover, in some α-thal2, Hb CS, Hb E traits, MCV and MCH are greater than 80 fL and 27 pg, respectively, which is similar to previous studies[5,17]. Hence, the use of MCV at less than 80 fL as the screening value for thalassemia did not cover all carriers and did not prevent Hb H and β-thal/Hb E disease resulting from those carriers. Both OF and MCV are effective in combination with the DCIP in hemoglobin typing and α-thalassemia analyses. Further more, the MCV-DCIP combination is slightly more sensitive.

In addition, of 94 Hb E traits, 29 (31.8%) were also positive for α-thalassemias, Hb CS and Hb PS. The most commonly found were α-thal 2 traits (10.6%), Hb CS traits (8.5%), α-thal 1 traits (5.3%) and Hb PS traits (3.2%). The combinations of Hb E traits with α-thal 1 may result in a decreased percentage of Hb E determined by automated LPLC. Previous studies suggest that the low level of Hb E in adult heterozygotes with α-thal1 trait results primarily from the greatly decreased assembly rate of alpha beta chain dimmers[21,32].

Regarding routine hemoglobin typing by HPLC or LPLC, the detection of Hb CS and Hb PS often fails because Hb CS and Hb PS are unstable variants with typical cathodic mobility. In of the present study, using DNA analyses, the prevalence of Hb CS traits and Hb PS traits is approximately 8% and 2%, respectively, similar to previous studies in Thai populations[2,31]. Though these carrier states show no clinical signs, the complex between a carrier state of a variety of thalassemic alleles results in a wide spectrum of clinical syndromes, exemplified by EA Bart's syndrome and Hb H-CS disease[4,33,34]. In conclusion, using only the OF and DCIP tests will cover all α-thalassemia$_1$ traits and α-thal traits; however, a combination of MCV and DCIP would yield more reliable results. Moreover, this observation suggests DNA analysis for α-thalassemias should be included in the program for the prevention and control of thalassemia in Thailand and possibly other countries in Southeast Asia, where α-thalassemias are highly prevalent.

Potential conflicts of interest

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ความชุกและลักษณะความผิดปกติของเม็ดเลือดแดงของโรคธาลัสซีเมียในตำบลท่ากระดาน จังหวัดฉะเชิงเทรา

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วัตถุประสงค์: *เพื่อศึกษาความชุก และลักษณะทางโลหิตวิทยาระดับโมเลกุลของโรคธาลัสซีเมีย ในประชากรตำบลท่ากระดาน จังหวัดฉะเชิงเทรา*

วัสดุและวิธีการ: *ศึกษาในประชากรจำนวน 266 คน ของหมู่ 19 บ้านนางาม จังหวัดฉะเชิงเทรา โดยทำการเก็บตัวอย่างเลือดแล้วนำมาตรวจกรองโรคธาลัสซีเมียด้วยการทดสอบ OF และ DCIP จากนั้นทำการตรวจเพิ่มเติมด้วย CBC, RBC indices, hemoglobin typing, Hb A_2 และ Hb E และตรวจหาความผิดปกติของยีนชนิด α-thalassemias ด้วยวิธี PCR โดยใช้ allele specific primers และวิธี Gap PCR*

ผลการศึกษา: *ความชุกของ α-thalassemia$_1$, α-thalassemia$_2$ และ β-thalassemia เท่ากับ 2.72%, 11.26% และ 0.97% ตามลำดับ และความชุกของฮีโมโกลบินผิดปกติชนิด Hb E, Hb Constant Spring และ Hb Pakse เท่ากับ 38.45%, 3.69% และ 0.78% ตามลำดับ ค่า MCV และ MCH ของผู้ที่เป็นพาหะของ β-thalassemia และ α-thalassemia$_1$ มีความแตกต่างอย่างมีนัยสำคัญกับคนปกติ นอกจากนี้พบว่าค่า MCV และ MCH ในผู้ที่เป็นพาหะของ α-thalassemia$_1$ ทุกราย มีค่าน้อยกว่า 75 fL และ 25 pg ดังนั้นการตรวจกรองโรคธาลัสซีเมียสามารถใช้ดัชนีชี้วัดความผิดปกติของเม็ดเลือดแดงเหล่านี้ได้*

สรุป: *ความชุกของโรคธาลัสซีเมียในประชากรที่ศึกษาครั้งนี้คล้ายคลึงกับการศึกษาที่ผ่านมา อย่างไรก็ตามการตรวจกรองโดยใช้การทดสอบ OF ร่วมกับ DCIP จะให้ค่าผลบวกปลอมมากกว่าการตรวจโดยใช้ MCV, MCH และ DCIP จึงจำเป็นต้องทำการตรวจยืนยันด้วยการวิเคราะห์ Hb typing และการใช้ MCV และ MCH เป็นตัวชี้วัดร่วมกับการทดสอบ DCIP ยังสามารถลดค่าใช้จ่ายในการตรวจได้ ซึ่งเหมาะสำหรับการตรวจกรองในประชากรจำนวนมาก*