Screening for Thalassemia and Hemoglobinopathy in a Rural Area of Thailand: A Preliminary Study

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The present study aimed to screen thalassemia and hemoglobinopathy in Baan Na-Ngam, Chachoengsao Province, Thailand. Blood samples were obtained from 266 volunteers; 105 males and 161 females aged 7 to 49 years. Blood samples screened for thalassemia combining the OF and modified DCIP precipitation tests. CBC, RBC indices, hemoglobin typing, HbA_2 and Hb E were determined. Combined OF and DCIP tests found that in normal subjects, 128 out of 155 were negative for both, 3 were -/+ pattern, 22 were +/- pattern and 2 was positive for both. Interestingly, one sample showed an abnormal hemoglobin pattern, which could not be determined by automated LPLC. Three β-thalassemia trait subjects were positive for only the OF test. For the Hb E trait, 57 out of 94 were -/+ pattern; 37 were positive for both tests. Moreover, 14 homozygous Hb E subjects were positive for both tests. The prevalence of β-thalassemia trait was 1.1%, Hb E trait was 35.3% and homozygous Hb E was 5.3%. Since DNA analysis was not performed, α-thalassemia$_1$ and α-thalassemia$_2$ traits cannot be excluded.

In conclusion, a combination of the OF and DCIP tests is suitable for preliminary screening for thalassemia and hemoglobinopathy. However, RBC parameters, hemoglobin typing and PCR analysis will provide more specific diagnosis, especially in α-thalassemias.

Keywords: *Screening tests, Thalassemia, Hemoglobinopathy, Thais*

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Thalassemia and hemoglobinopathy are the most common inherited disorders in Thailand and Southeast Asian countries[1-3]. The most important disorders are α-thalassemia and β-thalassemia, which are hemoglobin (Hb) Bart's hydrop fetalis, Hb H disease, homozygous β-thalassemia and β-thalassemia/ Hb E (β/E) disease. The prevalence rates of thalassemia and hemoglobinopathies

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are α-thalassemia 20-30 %, β-thalassemia 3-9 %, Hb E 13 % and Hb Constant Spring 1-8 %[3]. Presently, more than 10 million Thais carry the thalassemia gene for both disease and trait; approximately 600,000 have thalassemia disease. The severity of the disease varies from mild to severe anemia[4]. Therefore, in Thailand, the Ministry of Public Health has set up a ten-year program of prevention and control of thalassemia, not only to support the patients' quality of life but also to prevent thalassemia by reducing the number of new cases. 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 including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW)[5-14]. However, additional confirmatory tests such as hemoglobin typing for determination of Hb A_2, Hb E and DNA analysis are also needed[15]. A recent study in 358 Royal Thai Army (RTA) Medical cadets showed that the Hb E trait was the most frequent, 12.61 % and 12.0 %, followed by α-thalassemia$_1$ trait, 3.30 % and 4 % and β-trait, 1.50 % and 0 % in males and females, respectively. Interestingly, a mild form of β-thalassemia/ Hb E was found in one male RTA medical cadet[16]. However, in the northeastern part of Thailand, Cambodia and in Lao PDR, high incidences of Hb E (more than 50%) have been reported[1,2,17,18].

Baan Na-Ngam, Chachoengsao Province, is a remote rural area, 200 kilometers from Bangkok. Most of its population of 12,000 migrated from the rural northeastern part of Thailand. The majority of them are farmers, who are confined to their homes and rarely travel outside their province. The objective of the present study was to survey the prevalence of thalassemia and hemoglobinopathies in children and adults who live in this remote rural area of Thailand.

Material and Method

Subjects

Altogether 266 subjects were recruited from Moo 19, Baan Na-Ngam, Chachoengsao Province, Thailand. Three mL of peripheral blood anticoagulated with ethylenediamine tetraacetic acid (EDTA) 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 HbE 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 analyzer (Coulter Electronics, Hialeah, FL, USA).

Screening Test

The OF test was performed as described above[19,20]. A 0.34% buffer saline solution was prepared. A sample of 20 μL of whole blood was mixed with 5 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 the present study[19,20]. For the DCIP precipitation test, 20 μL of whole blood was added to 2 mL of a modified DCIP reagent, and the tube was incubated at 37°C for 15 minutes before 20 μL of stopping agent was added. Both tests were interpreted as negative or positive by visualization. Negative samples are clear, and positive samples

are cloudy. In the present study, suspicious samples with very little cloudiness were considered to be positive.

Hemoglobin Typing

Hemoglobin typing was conducted by automated low-pressure liquid chromatography; LPLC (Hb GOLD, Drew, U.K.)[21].

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 of various numerical parameters were calculated. Non-paired Student's *t*-test was used to compare the mean Hb, Hct, MCV, MCH, MCHC and RDW levels among the groups.

Results

Altogether 266 subjects were screened for thalassemia and hemoglobinopathy using the OF and DCIP tests. The CBC, RBC indices, hemoglobin typing, HbA_2 and Hb E were determined. The subjects were divided into four groups depending on the results of the OF and DCIP tests. First; subjects with negative results in both tests (-/-), second; subjects with a negative OF and a positive DCIP (-/+), third; subjects with a positive OF and a negative DCIP (+/-) and fourth; subjects with positive results in both tests (+/+), as shown in Table 1. Using a combination of the OF and the DCIP tests for preliminary screening for thalassemia and hemoglobinopathy, it was found that in normal subjects, 128 out of 155 were negative for both tests, 3 were -/+ pattern, 22 were +/- pattern and 2 was positive for both tests. Interestingly, one sample showed an abnormal hemoglobin pattern, which could not be determined by automated LPLC. For three β-thalassemia trait subjects, all were positive for only the OF test. In Hb E trait, 57 out of 94 were -/+ pattern and 37 were positive for both tests. Moreover, in 14 homozygous Hb E subjects, all were positive for both tests. From the present study, the prevalence of β-thalassemia trait was 1.1%, Hb E trait was 35.3% and homozygous Hb E was 5.3 %, respectively. It should be noted that α-thalassemia$_1$ and α-thalassemia$_2$ traits cannot be excluded in all subjects as DNA analysis was not performed.

The hematological analysis among various groups of subjects is shown in Table 2. The results reveal that the levels of Hb and Hct for β-thalassemia trait, Hb E trait and homozygous Hb E are lower than in normal subjects. The RBC indices, including MCV, MCH and MCHC, were also lower than in normal subjects, especially for homozygous Hb E, which was lower than

Table 1. The OF/DCIP pattern in 266 subjects

Type	No.	OF/DCIP			
		-/-	-/+	+/-	+/+
Normal*	155	128**	3	22	2
β-thalassemia trait*	3	0	0	3	0
Hb E trait*	94	0	57	0	37
Homozygous Hb E*	14	0	0	0	14
Total	266	128	60	26	52

* Not excluding α-thalassemia$_1$ and α-thalassemia$_2$ traits

** One sample showed abnormal hemoglobin pattern, which could not be determined by automated LPLC

Table 2. The Hb, Hct, RBC indices, RDW, HbA_2 and Hb E in 266 subjects

Test	Type			
	Normal*	β-thal trait*	Hb E trait*	Homozygous E*
	N=155	N=3	N=94	N=14
Hb (g/dL)	13.4±1.4	11.7±1.0	12.9±1.4	11.6±1.2
Hct (%)	40.0±3.8	36.7±2.7	38.9±3.9	36.1±3.1
MCV (fL)	86.2±7.7	68.8±3.0	79.5±5.3	65.8±5.7
MCH (pg/dL)	28.9±3.0	22.0±0.9	26.3±2.0	21.0±2.1
MCHC (g/dL)	33.5±1.0	32.0±0.7	33.1±0.8	31.9±0.8
RDW (%)	13.7±1.6	16.2±0.6	14.1±1.0	17.0±6.5
HbA2 (%)	2.5±0.4	6.2±0.7	-	-
Hb E (%)	-	-	28.4±2.9	87.7±3.7

N = Number tested

*p value < 0.05

β-thalassemia trait and Hb E trait. The RDW, representing the anisocytosis of red blood cells among all types, shows that for homozygous Hb E, the RDW was higher than β-thalassemia trait and Hb E trait (17.0 ± 6.5 %, 16.2 ± 0.6 % and 14.1 ± 1.0 % for homozygous Hb E, β-thalassemia trait and Hb E trait, respectively, compared to 13.7 ± 1.6 % in normal subjects). The level of Hb E is 87.7 ± 3.7 % for homozygous Hb E compared to 28.4 ± 2.9 % for Hb E trait (Table 2).

Table 3. The Hb, Hct, RBC indices, RDW, Hb A_2 and Hb E in different OF/DCIP patterns of normal and Hb E trait subjects

Test	Normal				Hb E trait	
	-/-	-/+	+/-	+/+	-/+	+/+
	(N=128)	(N=3)	(N=22)	(N=2)	(N=57)	(N=37)
Hb (g/dL)	13.6±1.3	13.4±1.5	12.1±1.4*	14.2±0.1	12.8±1.2*	13.0±0.4
Hct (%)	40.4±3.6	39.7±4.7	37.3±4.0*	43.2±0.7	38.7±3.5*	39.3±0.7
MCV (fL)	87.8±5.2	92.5±11.5	76.9±11.4*	80.3±18.1	79.5±3.9*	79.6±0.1*
MCH (pg/dL)	29.6±2.1	31.2±3.8	25.1±4.2*	26.7±6.2	26.3±1.5*	26.4±1.1*
MCHC (g/dL)	33.6±0.9	33.7±0.5	32.5±0.9*	33.3±0.2	33.1±0.8*	33.1±1.6*
RDW (%)	13.4±1.0	13.8±0.8	15.7±2.7*	14.0±1.8	13.8±0.7*	14.5±0.6*
Hb A_2 (%)	2.5±0.4	2.3±0.5	2.3±0.4*	2.4±0.2	-	-
Hb E (%)	-	-	-	-	29.2±1.7	27.2±0.1

N = Number tested

*p value < 0.05

RBC parameters in different OF/DCIP patterns of normal and Hb E subjects are summarized in Table 3. When comparing the values of Hb, Hct, MCV and MCH in four different OF/DCIP patterns of 155 normal subjects, it was found that only 22 subjects (+/-) showed lower levels of RBC parameters than the other patterns ($p<0.05$). In contrast, the RDW values were higher than those of the other normal subjects. Additionally, in 94 Hb E trait subjects, it was found that not only was there an abnormality of Hb E but also slightly low values of Hb, Hct ($p>0.05$) and RBC indices ($p<0.05$). Furthermore, in 14 homozygous E subjects, the Hb, Hct and RBC parameters were significantly lower than normal subjects ($p<0.05$).

Discussion

Routinely, in Thailand, the OF and DCIP tests are used as a screening protocol for thalassemia and hemoglobinopathy. However, the use of CBC and RBC indices obtained by automated blood cell analyzer have been added[12, 22, 23]. Individuals with low MCV (<80 fL) and MCH (<27 pg) usually have further investigation using electrophoresis or HPLC or LPLC or DNA analyses to identify α^0-thalassemia, β-thalassemia and Hb E carriers[24].

As expected, Hb E, which is prevalent among Southeast Asian populations, was the most common found in the present study; it was identified in 108 (40.6%) of 266 subjects. The β-thalassemia trait clearly was less common; it was found in only 3 (1.1%) of 266 subjects. Moreover, in the β-thalassemia group, abnormal RBC indices with increased percentages of the hemoglobin A_2 level were found and all were positive by the OF test. One study showed that the OF and/ or abnormal electrophoretic pattern were used as a screening test for thalassemia in Hellenic Army recruits[5]. The α-thalassemias cannot be excluded in all subjects, further PCR analysis studies are suggested. Regarding the OF/DCIP test results, 3 and 22 false positive results by the OF and DCIP were found in 155 normal subjects. However, this is not so important because the major concern of screening is to avoid false negative results[24]. Interestingly, excellent results of the DCIP for Hb E were obtained, which is similar to previous studies[24, 25]. This should reduce the need for further hemoglobin typing and PCR analysis. Thus, a combination of the OF and DCIP tests is an appropriate preliminary measure that can be used at any small community hospital or in communities with limited facilities and economic resources.

Additionally, one normal subject with normal RBC parameters was negative for the OF and DCIP tests. The abnormal hemoglobin was found, but could not be determined by automated LPLC. PCR analysis is needed to identify specific hemoglobin type.

In conclusion, these findings suggest that a combination of the OF and DCIP tests is suitable for a preliminary screening for thalassemia and hemoglobinopathy. However, RBC parameters, hemoglobin typing and PCR analysis will provide more specific diagnosis especially in α-thalassemias.

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References

1. Wasi P, Pootrakul S, Pootrakul P, Pravatmuang P, Winichagoon P, Fucharoen S. Thalassemia in Thailand. Ann N Y Acad Sci 1980; 344: 352-63.

2. Fucharoen S, Winichagoon P. Hemoglo binopathies in Southeast Asia. Hemoglobin 1978; 11: 65-88.
3. Fucharoen S, Winichagoon P, Siritanaratkul N, Chowthaworn J, Pootrakul P. Alpha- and beta- thalassemia in Thailand. Ann N Y Acad Sci 1998; 850: 412-4.
4. Wasi P, Pootrakul P, Fucharoen S, Winichagoon P, Wilairat P, Promboon A. Thalassemia in Southeast Asia: determination of different degrees of severity of anemia in thalassemia. Ann N Y Acad Sci 1985; 445: 119-26.
5. Tegos CN, Voutsadakis AJ, Karmas PA. Diagnostic strategy for thalassemias and other hemoglobinopathies: a program applied to the Hellenic Army recruits. Mil Med 1992; 157: 183-5.
6. Wiwanitkit V, Suwansaksri J, Paritpokee N. Combined one-tube osmotic fragility (OF) test and dichlorophenol-indolphenol (DCIP) test screening for hemoglobin disorders, an experience in 213 Thai pregnant women. Clin Lab 2002; 48: 525-8.
7. Jindadamrongwech S, Wisedpanichkij R, Bunyaratvej A, Hathirat P. Red cell parameters in alpha-thalassemia with and without beta-thalassemia trait or hemoglobin E trait. Southeast Asian J Trop Med Public Health 1997; 28: 97-9.
8. Lafferty JD, Crowther MA, Ali MA, Levine M. The evaluation of various mathematical RBC indices and their efficacy in discrimination between thalassemic and non-thalassemic microcytosis. Am J Clin Pathol 1996; 106: 201-5.
9. Lima CS, Reis AR, Grotto HZ, Saad ST, Costa FF. Comparison of red cell distribution width and a red cell discriminant function incorporating volume dispersion for distinguishing iron deficiency from beta thalassemia trait in patients with microcytosis. Rev Paul Med 1996; 114: 1265-9.
10. Fucharoen S, Winichagoon P, Piankijagum A. Standardization on laboratory diagnosis of thalassemia and abnormal hemoglobin. Southeast Asian J Trop Med Public Health 1999; 30 (Suppl 3): 90-8.
11. Ma ESK, Chan AYY, Ha SY, Chan LC. Thalassemia screening on red blood cell indices in the Chinese. Haematologica 2001; 86: 1310-1.
12. Chan LC, Ma SK, Chan AYY, Ha SY, Wayes JS, Lau YL. Should we screen for globin gene mutations in blood samples with mean corpuscular volume (MCV) greater than 80 fL in areas with a high prevalence of thalassemia? J Clin Pathol 2001; 54: 317-20.
13. Silvestroni E, Bianco I. A highly cost effective method of mass screening for thalassemia. BMJ 1983; 286: 1007-9.
14. Kattamis C, Efremov G, Pootrakul S. Effectiveness of one tube osmotic fragilty test screening in detecting β-thalassemia trait. J Med Genet 1981; 18: 266-70.
15. Chan AY, So CK, Chan LC. Comparison of the Hb H inclusion test and a PCR test in routine screening for alpha thalassaemia in Hong Kong. J Clin Pathol 1996; 49: 411-3.
16. Nathalang O, Arnutti P, Nillakupt K. Thalassemia screening among Royal Thai Army medical cadets. Mil Med 2004; 169: 274-76.
17. Na-Nakorn S, Wasi P. The distribution of hemoglobin E: hemoglobin E triangle in Southeast Asia. J Med Assoc Thai 1972; 61: 65-8.
18. Fucharoen P, Fucharoen S, Sanchaisuriya K, Sae-ung N, Suyasunanond A, Sriwilai P, et al. Frequency distribution and haplotype heterogeneity of β^E - globin gene among eight minority groups of Northeast Thailand.

Hum Hered 2002; 53: 18-22.
19. Fucharoen G, Kanta K, Pratumchat R, Yimprasert U, Paikapet K, Surin U, et al. Thalassemia and iron deficiency among OF-test and KKU-DCIP-Clear screening positive population. [in Thai] Thai J Hematol Transfus Med 1999; 9: 111-8.
20. Panyasai S, Sringam P, Fucharoen G, Sanchaisuriya K, Fucharoen S. A simplified screening for α-thalassemia$_1$ (SEA type) using a combination of a modified osmotic fragility test and a direct PCR on whole blood cell lysates. Acta Haematol 2002; 108: 74-8.
21. Sanguansermsri T, Chomchuen S, Steger HF. Diagnosis of severe thalassemia trait using the low pressure liquid chromatography Hb Gold Drew® System. Bull Chiang Mai Assoc Med Sci 1999; 32: 144-9.
22. The thalassemia working party of the BCSH general haematology task force. Guidelines for investigation of the α-thalassemia and β-thalassemia traits. J Clin Pathol 1994; 47: 289-95.
23. A working party of the general haematology task force of the British committee for standards in haematology. The laboratory diagnosis of haemoglobinopathies. Br J Haematol 1998; 101: 783-92.
24. Fucharoen G, Sanchaisuriya K, Sae-ung N, Dangwibul S, Fucharoen S. A simplified screening strategy for thalassemia and haemoglobin E in rural communities in Southeast Asia. Bull World Health Organ 2004; 82 : 364-72.
25. Sanchaisuriya K, Fucharoen S, Fucharoen G, Ratanasiri T, Sanchaisuriya P, Changtrakul Y, Ukosanakarn U , Ussawapark W, Schelp FP. A reliable screening protocol for thalassemia and hemoglobinopathies in pregnancy: an alternative approach to electronic blood cell counting. Am J Clin Pathol 2005; 123: 113-8.

การตรวจกรองธาลัสซีเมียและฮีโมโกลบินผิดปกติในคนไทยที่อยู่ในชนบท

อ้อยทิพย์ ณ ถลาง, กมลทิพย์ นิลคุปต์, ภัสรา อาณัติ, ธนิตย์ บุญสิริ, สุธี พานิชกุล, วิโรจน์ อารีย์กุล

การศึกษานี้ได้ตรวจกรองหาพาหะและโรคธาลัสซีเมียรวมทั้งภาวะฮีโมโกลบินผิดปกติในประชากรบ้านนางาม จังหวัดฉะเชิงเทรา โดยได้เจาะเลือดอาสาสมัครจำนวน 266 คน เป็นชาย 105 คน และหญิง 161 คน ช่วงอายุตั้งแต่ 7-49 ปี ทำการตรวจกรองธาลัสซีเมียโดยใช้การทดสอบ OF และ modified DCIP แล้วทำการตรวจเพิ่มเติมด้วย CBC, RBC indices, hemoglobin typing, Hb A_2 และHb E จากผลการตรวจกรองด้วยวิธี OF และ DCIP พบว่า ในกลุ่มคนปกติ 155 ราย มี 128 ราย ให้ผลลบกับทั้ง 2 วิธี 3 รายให้ผลเป็น -/+ 22 รายให้ผล +/- และมี 2 รายให้ผลบวกกับทั้ง 2 วิธี นอกจากนี้พบว่า 1 ราย ที่ให้ผลฮีโมโกลบินผิดปกติจากการตรวจด้วยเครื่องอัตโนมัติ LPLC แต่ยังไม่สามารถสรุปชนิดของฮีโมโกลบินได้ สำหรับกลุ่ม β-thalassemia trait 3 ราย ให้ผลบวกกับวิธี OF เท่านั้น ส่วนกลุ่ม Hb E trait 94 ราย พบว่า 57 ราย ให้ผลเป็น -/+ และอีก 37 รายให้ผลบวกกับทั้ง 2 วิธี และในกลุ่ม homozygous Hb E 14 ราย พบว่าให้ผลบวกกับทั้ง 2 วิธี จากการศึกษาครั้งนี้พบว่าความชุกของ β-thalassemia trait คือ 1.1%, Hb E trait 35.3 % และ homozygous Hb E 5.3% แต่เนื่องจากไม่ได้ทำการวิเคราะห์ด้วยวิธี ดีเอ็นเอ จึงยังไม่สามารถแยกชนิดของ α-thalassemia$_1$ และ α-thalassemia$_2$ trait ได้

โดยสรุปพบว่าการใช้วิธี OF ร่วมกับ DCIP สามารถใช้ในการตรวจกรองเบื้องต้นของธาลัสซีเมียและฮีโมโกลบินผิดปกติได้ แต่อย่างไรก็ตามการใช้ผล RBC parameters, hemoglobin typing และการตรวจ PCR จะช่วยในการวินิจฉัยให้ถูกต้องและแม่นยำยิ่งขึ้น