A simplified screening strategy for thalassaemia and haemoglobin E in rural communities in south-east Asia

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Objective To evaluate a simple screening strategy for thalassaemia and haemoglobin (Hb) E in a prevention and control programme for thalassaemia in rural communities with limited resources.

Methods Blood samples from 301 Thai–Khmer participants were screened for thalassaemia and Hb E using a combined modified one-tube osmotic fragility (OF) test and a modified dichlorophenolindophenol (DCIP) precipitation test. Results were evaluated with standard haematological analyses including erythrocyte indices, Hb typing and quantification and polymerase chain reaction (PCR) analysis of α-globin and β-globin genes.

Findings Participants were divided into four groups according to the results of the combined tests. Altogether, 104 of 301 participants (34.6%) had negative results on both tests; 48 (15.9%) were positive on the OF test but not the DCIP test; 40 (13.3%) were negative on the OF test but positive on DCIP test; and 109 (36.2%) were positive on both tests. No carrier of clinically significant forms of thalassaemia (α^o-thalassaemia, β-thalassaemia) or Hb E was found among the group that had negative results for both tests. All participants with Hb E had positive DCIP tests. Carriers of α^+-thalassaemia or Hb Constant Spring could generate either positive or negative OF test results but they all had negative DCIP tests. Using both tests as a preliminary screening for the three important groups of carriers gave a sensitivity of 100% and a specificity of 69.8%. The positive predictive value of the combined test was 77.2%. The negative predictive value was 100%. Further evaluation of the screening system by local staff at three community hospitals found a sensitivity of 98.1–100% and a specificity of 65.4–88.4% with positive predictive values of 75.0–86.9% and negative predictive values of 98.1–100%.

Conclusion A combined test using OF and DCIP could be used as an effective preliminary screening alternative to an electronic blood cell count for identifying carriers with α^o-thalassaemia, β-thalassaemia and Hb E. The strategy should prove useful for population screening in prevention and control programmes in rural communities in south-east Asia where laboratory facilities and economic resources are limited.

Keywords Thalassemia/diagnosis/blood; Hemoglobin E/diagnostic use; Osmotic fragility; Precipitin tests; 2,6-Dichloroindophenol/diagnostic use; Erythrocyte indices; Carrier state/blood; Rural population; Cambodia; Lao People's Democratic Republic; Thailand; South-East Asia. (*source: MeSH, NLM*).

Mots clés Thalassémie/diagnostic/sang; Hémoglobine E/usage diagnostique; Sensibilité choc osmotique; Réaction précipitation; Dichloro-2,6 indophénol/usage diagnostique; Indice hématie; Porteur germes/sang; Population rurale; Cambodge; République démocratique populaire lao; Thaïlande; Asie sud est (*source: MeSH, INSERM*).

Palabras clave Talasemia/diagnóstico/sangre; Hemoglobina E/uso terapéutico; Fragilidad osmotica; Tests de precipitina; 2,6-Dichloroindofenol/uso terapéutico; Indices de eritrocitos; Portador/sangre; Población rural; Población rural; Camboya; República Democrática Popular Lao; Tailandia; Asia Sudoriental (*fuente: DeCS, BIREME*).

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Introduction

Thalassaemia and haemoglobinopathy are the most common inherited disorders among humans, and they represent a major public health problem in many areas of the world, including south-east Asia (*1*). The most important disorders are α-thalassaemia and β- thalassaemia. Among the structural haemoglobin (Hb) variants, Hb E ($\alpha_2\beta_2^{26glu\text{-}lys}$) is the most common, especially in the north-eastern part of Thailand, and in Cambodia and Laos (*2*, *3*). A high incidence of Hb E (more than 50%) has been reported in many groups with a Mon-Khmer linguistic affiliation (*4*).

In these areas, the prime targets of prevention and control of severe thalassaemia are homozygous α^o-thalassaemia (Hb

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Bart's hydrops fetalis syndrome), homozygous β-thalassaemia and β-thalassaemia–Hb E disease (*5*, *6*). The aim of screening for thalassaemia and Hb disorders is to offer carrier testing to every member of the population, ideally before they have children, in order to identify carrier couples and inform them of the risk and their options. The people targeted by screening are therefore carriers of α^{o}-thalassaemia, β-thalassaemia and Hb E.

As a general guideline, primary screening for all forms of thalassaemia involves using an electronic blood-cell counter to provide accurate erythrocyte indices. Individuals who have hypochromic microcytosis with mean corpuscular volume (MCV) below 80 fl or mean corpuscular Hb (MCH) below 27 pg should be investigated further using Hb electrophoresis or high-performance liquid chromatography (HPLC) (*7*, *8*). This, however, can be problematic in rural areas in south-east Asia where the expense usually precludes the possibility of electronic blood-cell counting. We evaluated a cheaper alternative screening method using a combination of a modified one-tube osmotic fragility (OF) test (*9*, *10*) and a modified dichlorophenolindophenol (DCIP) precipitation test (*11*). This method could be used in any primary health care setting where a programme of prevention and control is needed.

Methods

Participants and haematological analysis

One ml of peripheral blood anticoagulated with ethylenediaminetetraacetic acid (EDTA) was obtained from 301 healthy unrelated Thai–Khmer individuals (age range, 8–30 years) living in the provinces of Surin and Burirum in north-eastern Thailand. Informed consent was obtained, and each participant received a written report of the results of the screening tests.

After collection, all samples were immediately screened for thalassaemia using the modified one-tube OF test. They were screened for Hb E using the modified DCIP precipitation test. The modified DCIP test kit, which uses a clear reagent, was developed in our laboratory (*12*, *13*). All blood samples were put on ice and transferred within six hours to the Faculty of Associated Medical Sciences, Khon Kaen University, for determination of erythrocyte indices using the Coulter-STKS automated blood-cell counter (Coulter Electronics, Hialeah, FL, USA).

Screening test

The OF test was performed as described previously (*9*, *10*) but with slight modification. Instead of using a 0.36% buffered saline solution, we used a 0.34% saline solution in the same buffer. This modification can significantly reduce the number of false-positive samples without changing the sensitivity to α^{o}-thalassaemia and β-thalassaemia (*13*). In the reagent kit, the 0.34% buffered saline is prepared for each screening in a 13 mm x 75 mm capped plastic tube. A sample of 20 µl whole blood is mixed with 2 ml of the saline solution in the test tube and left at room temperature for 15 minutes before being interpreted. For the DCIP precipitation test, 20 µl whole blood is added to 2 ml of a modified DCIP reagent, and the tube is incubated at 37 °C for 15 minutes before 20 µl of stopping agent is added. Both tests are interpreted as negative or positive by visualization. Negative samples are clear and positive samples are cloudy. For this study, suspicious samples with very little cloudiness were considered to be positive.

Hb analysis

Types and levels of Hb fractions were determined using an automated low-pressure liquid chromatography (LPLC) Hb Analyzer (Hb Gold, Drew Scientific Ltd., Barrow-in-Furness, Cumbria, England) and standard cellulose acetate electrophoresis.

DNA analysis

Genomic DNA was prepared from peripheral blood leukocytes using a standard method. Identification of α^{o}-thalassaemia (SEA deletion), α^{+}-thalassaemia (3.7 kb and 4.2 kb deletion) and Hb Constant Spring (Hb CS) gene were performed using allele-specific polymerase chain reaction (PCR) as described elsewhere (*13*–*15*). Common β-thalassaemia mutations in Thailand were examined using PCR and related techniques described previously (*16*, *17*).

Validation of the screening system

To validate the screening procedure at local hospitals, a combined screening was performed by local staff at three additional community hospitals in the Roi Et province in north-eastern Thailand. Hospital A screened 122 additional participants; Hospital B screened 120; and Hospital C screened 128. The results of these tests were evaluated using standard methods at the authors' laboratory, and the screening efficiency was calculated accordingly.

Results

The results of screening for thalassaemia and Hb E using the two test kits, the haematological analysis and the α-globin and β-globin genotypings of the 301 Thai–Khmer participants are summarized in Table 1. Participants were divided into four groups depending on their results:

- Participants with negative results in both tests (-/-)
- Participants with a negative OF and a positive DCIP (-/+)
- Participants with a positive OF and a negative DCIP (+/-)
- Participants with positive results on both tests (+/+).

Among the 104 (34.6%) participants who had negative results on both tests (-/- group), relatively normal haematological parameters including MCV, MCH, mean corpuscular Hb concentration (MCHC) and red blood cell distribution width (RDW) were found. Further Hb and DNA analyses identified no α^{o}-thalassaemia, β-thalassaemia or Hb E carriers in this group.

All 48 participants (15.9%) with the +/- pattern had Hb A2A with normal Hb A2 concentrations. Three α^{o}-thalassaemia carriers with the SEA deletion were identified in this group. The erythrocyte indices including MCV and MCH values of these three carriers were lower than normal (mean MCV 69.1, SD 5.3 fl; mean MCH 21.4, SD 2.0 pg). The α^{o}-thalassaemia carriers had positive OF tests, but the α^{+}-thalassaemia carriers could generate either a positive result or a negative result.

All 40 cases (13.3%) with the -/+ pattern of results are Hb E carriers with or without α^{+}-thalassaemia, indicating an excellent sensitivity and specificity when the DCIP test is used to detect Hb E. We found that the levels of Hb E in pure Hb E carriers and Hb E carriers with α^{+}-thalassaemia were not significantly different (mean Hb E 28.8, SD 2.6 % vs mean Hb E 27.0, SD 1.0; and mean Hb E 28.2, SD 2.1 %). The erythrocyte indices in this group are no different from those in the group that had negative results in both tests. Therefore, it is possible that the DCIP test is more appropriate than erythrocyte indices in screening for Hb E carriers in this population.

Table 1. **Results of the combined one-tube osmotic fragility test (OF) and modified dichlorophenolindophenol precipitate (DCIP) test, haematological analyses and globin genotypes for 301 Thai–Khmer participants.** Data presented as mean (SD) or raw data where appropriate

Results of combined test (OF/DCIP)[a]	No. of participants	Hb (g/dl)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	Hb Type	Hb A2/E (%)	α - genotype[b]	β - genotype
	68	13.0 (1.6)	40.3 (4.9)	88.2 (6.0)	28.5 (1.8)	32.3 (1.4)	13.5 (1.2)	A_2A	2.6 (0.3)	α α/α α	β^A/β^A
- / -	27	11.7 (1.6)	31.4 (5.0)	86.7 (4.7)	27.3 (3.2)	31.4 (3.4)	13.6 (1.0)	A_2A	2.4 (0.3)	$-\alpha^{3.7}/\alpha\ \alpha$	β^A/β^A
	2	10.0, 12.1	36.5, 37.4	79.9, 82.1	21.9, 24.3	27.4, 28.6	16.8, 16.1	A_2A	2.4, 2.0	$-\alpha^{4.2}/\alpha\ \alpha$	β^A/β^A
	5	12.6 (2.1)	38.0 (5.0)	79.1 (2.1)	25.7 (2.1)	32.5 (2.2)	14.1 (1.1)	A_2A	2.1 (0.3)	$\alpha^{CS}\ \alpha/\alpha\ \alpha$	β^A/β^A
	2	9.8, 8.0	34.7, 30.4	80.5, 90.2	22.7, 23.7	28.0, 26.3	15.3, 13.3	A_2A	1.6, 2.6	$\alpha^{CS}\ \alpha/-\alpha^{3.7}$	β^A/β^A
Total	**104**										
	14	13.6 (1.9)	41.6 (4.7)	86.6 (8.8)	28.3 (2.8)	32.7 (1.5)	14.4 (1.4)	A_2A	2.4 (0.4)	α α/α α	β^A/β^A
	16	13.5 (1.9)	42.8 (6.5)	83.4 (7.2)	26.7 (2.5)	31.5 (1.6)	14.7 (1.5)	A_2A	2.3 (0.3)	$-\alpha^{3.7}/\alpha\ \alpha$	β^A/β^A
+ / -	1	13.8	42.2	83.3	27.3	32.7	14.2	A_2A	2.5	$-\alpha^{4.2}/\alpha\ \alpha$	β^A/β^A
	1	10.2	35.7	83.0	23.7	28.6	14.6	A_2A	2.5	$\alpha^{CS}\ \alpha/\alpha\ \alpha$	β^A/β^A
	11	11.5 (1.1)	38.4 (3.4)	74.2 (2.6)	22.3 (1.1)	30.1 (1.8)	15.9 (1.1)	A_2A	2.2 (0.2)	$-\alpha^{3.7}/-\alpha^{3.7}$	β^A/β^A
	1	13.1	40.9	68.8	22.0	32.0	16.3	A_2A	2.3	$-\alpha^{4.2}/-\alpha^{4.2}$	β^A/β^A
	1	10.8	41.1	86.9	22.8	26.3	15.0	A_2A	2.4	$\alpha^{CS}\ \alpha/-\alpha^{3.7}$	β^A/β^A
	3	10.4 (1.6)	33.7 (3.8)	69.1 (5.3)	21.4 (2.0)	30.9 (1.2)	17.8 (2.2)	A_2A	2.3 (0.6)	$--^{SEA}/\alpha\ \alpha$	β^A/β^A
Total	**48**										
	26	12.3 (1.5)	38.2 (6.4)	79.8 (4.10)	25.9 (2.9)	31.6 (1.4)	13.7 (1.8)	EA	28.8 (2.6)	α α/α α	β^A/β^E
- / +	7	12.4 (1.3)	40.0 (4.2)	83.0 (4.1)	25.8 (1.1)	31.1 (1.5)	13.7 (1.1)	EA	27.0 (1.0)	$\alpha^{CS}\ \alpha/\alpha\ \alpha$	β^A/β^E
	6	12.8 (2.4)	40.9 (8.8)	83.0 (5.8)	26.1 (0.8)	31.5 (2.0)	13.3 (1.0)	EA	28.2 (2.1)	$-\alpha^{3.7}/\alpha\ \alpha$	β^A/β^E
	1	14.5	44.1	84.8	28.0	33.0	14.1	EA	27.6	$-\alpha^{3.7}/-\alpha^{3.7}$	β^A/β^E
Total	**40**										
	2	8.8, 9.0	29.3, 28.4	60.4, 63.7	18.1, 19.7	29.9, 28.7	23.3, 22.6	A_2AH	1.3 , 1.4	$--^{SEA}/-\alpha^{3.7}$	β^A/β^A
	43	13.2 (2.1)	41.5 (6.6)	80.6 (5.1)	25.7 (1.7)	31.8 (1.1)	14.9 (2.2)	EA	29.3 (1.6)	α α/α α	β^A/β^E
	31	12.1 (1.7)	38.5 (5.8)	81.3 (5.1)	25.6 (1.9)	31.5 (1.6)	15.3 (2.3)	EA	26.9 (2.8)	$-\alpha^{3.7}/\alpha\ \alpha$	β^A/β^E
+ / +	1	12.1	36.4	78.2	25.1	31.5	16.1	EA	26.8	$-\alpha^{4.2}/\alpha\ \alpha$	β^A/β^E
	2	8.5, 13.0	27.3, 42.6	64.1, 72.2	19.9, 22.0	31.0, 30.4	19.7, 14.8	EA	18.8, 21.4	$\alpha^{CS}\ \alpha/-\alpha^{3.7}$	β^A/β^E
	3	10.9 (1.5)	34.5 (5.6)	66.2 (8.3)	21.1 (2.1)	31.9 (1.3)	17.0 (4.2)	EA	21.4 (0.4)	$--^{SEA}/\alpha\ \alpha$	β^A/β^E
	1	9.2	28.0	56.6	18.7	33.0	21.8	EA Bart's	15.9	$--^{SEA}/-\alpha^{3.7}$	β^A/β^E
	1	7.9	32.7	67.2	14.8	22.0	19.3	EA Bart's	16.2	$--^{SEA}/\alpha^{CS}\ \alpha$	β^A/β^E
	12	10.7 (0.7)	34.1 (1.9)	66.9 (3.0)	20.9 (0.9)	31.2 (0.8)	16.1 (1.0)	EE	87.4 (1.5)	α α/α α	β^E/β^E
	8	11.3 (0.7)	36.7 (2.7)	66.8 (1.6)	21.3 (2.0)	30.9 (0.6)	17.0 (0.6)	EE	85.3 (3.3)	$-\alpha^{3.7}/\alpha\ \alpha$	β^E/β^E
	2	10.1, 11.3	31.7, 336	74.6, 72.5	23.9, 22.8	31.9, 32.1	17.1, 17.4	EE	83.0 , 82.4	$\alpha^{CS}\ \alpha/\alpha\ \alpha$	β^E/β^E
	1	11.3	34.6	75.3	24.2	32.7	17.2	EE	89.0	$-\alpha^{3.7}/-\alpha^{3.7}$	β^E/β^E
	1	10.9	35.6	71.2	21.8	30.6	13.8	EE	85.0	$\alpha^{CS}\ \alpha/-\alpha^{3.7}$	β^E/β^E
	1	8.3	26.6	58.6	18.4	31.4	29.3	EF	61.4	α α/α α	β^{th}/β^E
Total	**109**										

[a] Results indicated as positive test (+) or negative test (-).

[b] Alleles are represented as follows: $-\alpha^{3.7}$ = 3.7 kb α^+-thalassaemia, $-\alpha^{4.2}$ = 4.2 kb α^+-thalassaemia, $\alpha^{CS}\ \alpha$ = Hb Constant Spring. $--^{SEA}$ = SEA deletion α^o-thalassaemia, β^E = Hb E, and β^{th} = β-thalassaemia.

Among the 109 (36.2%) participants who had positive results on both tests, we found various thalassaemias and haemoglobinopathies. All but two participants with Hb H disease had Hb E, either as Hb E heterozygote or homozygote with and without α-thalassaemia. This indicates that Hb H could generate false-positive results when the DCIP test is used for Hb E. The erythrocyte indices in this group were comparatively lower than in other groups. In addition to the two participants with Hb H disease, other forms of thalassaemia diseases were found, including two cases of EA Bart's disease and one case of β-thalassaemia–Hb E disease. Further PCR analysis of the β-globin gene revealed that the β-thalassaemia mutation in the latter was caused by an AAG to TAG mutation at codon 17 (data not shown). In Thailand, this is a common β-thalassaemia mutation (*16*). Eighty Hb E heterozygotes and 24 Hb E homozygotes were found in this group. While no α^{o}-thalassaemia was detected among the homozygotes, we detected three cases of double heterozygotes for Hb E and α^{+}-thalassaemia (SEA type). The levels of Hb E in these three cases were lower than those usually observed in individuals with the pure Hb E trait (mean Hb E 21.4 , SD 0.4 % vs mean Hb E 29.3, SD 1.6 %) (*18*).

We unexpectedly found 23 genotypes in this limited survey (Table 2). This data shows the heterogeneity of thalassaemia and haemoglobinopathies in this region and the effectiveness of the screening strategy. Using the data in Table 1, we found that the DCIP test had 100% sensitivity and 98.7% specificity for Hb E with 98.6% positive predictive value and 100% negative predictive value. A false-positive test was seen only in the two participants who had Hb H disease. Table 3 shows that using a combination of the modified OF test and the DCIP test for preliminary screening had 100% sensitivity and 69.8% specificity with a positive predictive value of 77.2% and a negative predictive value of 100% for the three groups of clinically important thalassaemia carriers, namely those with α^{o}-thalassaemia, β-thalassaemia and Hb E.

Table 4 and Table 5 show the results of the screening procedure carried out by staff at three community hospitals on three additional groups of participants. The prevalence of thalassaemia and haemoglobinopathies are summarized in Table 4. Table 5 shows that by using this combination of tests, all three community hospitals could screen efficiently for the three groups of carriers (sensitivity 98.1–100%; specificity 65.4–88.4%; positive predictive value 75.0–86.9%; negative predictive value 98.1–100%). The values found were quite similar to those of the preliminary screening results shown in Table 3.

Discussion

The screening protocol for thalassaemia usually relies on using a complete blood cell count with erythrocyte indices obtained using an automated blood-cell counter (*7*, *8*). Individuals with low MCV (< 80 fl) and MCH (< 27 pg) usually have further investigation using electrophoresis or HPLC or DNA analysis to identify α^{o}-thalassaemia, β-thalassaemia and Hb E carriers. This screening protocol, carried out for the purpose of offering genetic counselling and prenatal diagnosis, is straightforward, accurate and can effectively detect the majority of carriers with α^{o}-thalassaemia and β-thalassaemia (*19*, *20*). However, many

Table 2. **Prevalence of thalassaemias and haemoglobinopathies by genotype among 219 of 301 participants**

Genotype	No. of participants
Heterozygous α^{+}-thalassaemia (3.7 kb deletion)	43 (14.3)[a]
Heterozygous α^{+}-thalassaemia (4.2 kb deletion)	3 (1.0)
Heterozygous Hb CS	6 (2.0)
Heterozygous α^{o}-thalassaemia (SEA type)	3 (1.0)
Compound heterozygous for Hb CS and α^{+}-thalassaemia (3.7 kb type)	3 (1.0)
Homozygous α^{+}-thalassaemia (3.7 kb type)	11 (3.6)
Homozygous α^{+}-thalassaemia (4.2 kb type)	1 (0.3)
Heterozygous Hb E	69 (22.9)
Double heterozygous for Hb E and α^{+}-thalassaemia (3.7 kb type)	37 (12.3)
Double heterozygous for Hb E and α^{+}-thalassaemia (4.2 kb type)	1 (0.3)
Double heterozygous for Hb E and Hb CS	7 (2.3)
Double heterozygous for Hb E and α^{o}-thalassaemia (SEA type)	3 (1.0)
Heterozygous Hb E with homozygous α^{+}-thalassaemia (3.7 kb type)	1 (0.3)
Triple heterozygous for Hb E, α^{o}-thalassaemia and α^{+}-thalassaemia (3.7 kb type)	1 (0.3)
Triple heterozygous for Hb E, α^{o}-thalassaemia and Hb CS	1 (0.3)
Triple heterozygous for Hb E, α^{+}-thalassaemia (3.7 kb type) and Hb CS	2 (0.7)
Homozygous Hb E	12 (4.0)
Homozygous Hb E with heterozygous α^{+}-thalassaemia (3.7 kb type)	8 (2.7)
Homozygous Hb E with heterozygous Hb CS	2 (0.7)
Homozygous Hb E with homozygous α^{+}-thalassaemia (3.7 kb type)	1 (0.3)
Homozygous Hb E with Hb CS and α^{+}-thalassaemia (3.7 kb type)	1 (0.3)
Compound heterozygous for α^{o}-thalassaemia and α^{+}-thalassaemia (3.7 kb type) (Hb H disease)	2 (0.7)
β-thalassaemia–Hb E disease	1 (0.3)
Total	**219 (72.7)**

[a] Values in parentheses are percentages.

Table 3. **Efficiency of screening using the combined one-tube osmotic fragility test (OF) and modified dichlorophenolindophenol precipitate (DCIP) test for 301 Thai–Khmer participants tested for three forms of thalassaemia: α^o-thalassaemia, β-thalassaemia and Hb E.** See text for information on sensitivity, specificity, positive predictive value and negative predictive value

	Hb and DNA analysis		
OF/DCIP screening[a]	**With α^o-thalassaemia, β-thalassaemia or Hb E**	**Without α^o-thalassaemia, β-thalassaemia or Hb E**	**Total**
Positive tests			197
+/-	3	45[b]	
-/+	40	0	
+/+	109	0	
Negative tests			
- / -	0	104	104
Total	**152**	**149**	**301**

[a] Results indicated as positive test (+) or negative test (-).
[b] 31 of 45 were positive for α^+-thalassaemia and/or $\alpha^{Constant\ Spring}$ alleles by PCR analysis.

Table 4. **Prevalence of thalassaemia and haemoglobinopathies identified among 370 additional participants screened at three community hospitals**

Genotypes	**Hospital**			**No.**
	A	**B**	**C**	
Heterozygous α^o-thalassaemia	2	3	6	11 (2.9)[a]
Heterozygous β-thalassaemia	0	1	1	2 (0.5)
Heterozygous Hb E	35	40	57	132 (35.7)
Double heterozygous for Hb E and α^o-thalassaemia	0	4	3	7 (1.9)
Homozygous Hb E	9	2	9	20 (5.4)
Homozygous Hb E with α^o-thalassaemia	1	0	0	1 (0.3)
Hb H disease	2	0	0	2 (0.5)
α/β-thalassaemia disease (EA Bart's disease)	1	0	0	1 (0.3)
β-thalassaemia–Hb E	3	2	0	5 (1.4)
Normal or non-clinically significant haemoglobinopathy	69	68	52	189 (51.1)
Total	**122**	**120**	**128**	**370 (100)**

[a] Values in parentheses are percentages.

Hb E carriers will be missed when these cut-off values are used (*21*, *22*). Additionally, the need for an expensive automated blood-cell counter to determine erythrocyte indices could render the application of such a protocol unfeasible for a large-scale screening programme in south-east Asian communities which have a high prevalence of thalassaemia and haemoglobinopathy but that have limited facilities and economic resources.

Thool et al. (*23*) have had success with the naked-eye single-tube red-cell osmotic fragility test (NESTROFT) using 0.36% buffered saline to screen for β-thalassaemia carriers in India. We have now described another practical screening strategy in which the costs can be reduced considerably by using a combination of a modified one-tube OF test and a DCIP test. The OF test was used to identify individuals who may be carriers of α^o-thalassaemia or β-thalassaemia. The DCIP test was used to detect those with Hb E. Participants in this study were Thai–Khmer individuals from north-eastern Thailand. There is a high prevalence of thalassaemia and Hb E in this group (Table 2). Using this combination of tests for this population gave excellent results (Table 1).

There were no carriers of α^o-thalassaemia, β-thalassaemia or Hb E among the 104 participants who had negative results on both tests. This indicates that there would be no false negatives for these important groups of carriers. We also found that participants who were carriers of α^+-thalassaemia and Hb E could have either positive or negative results with the OF test. Hence some of the α^+-thalassaemia carriers were found in the group that had negative results for both tests, and some were found in the group that had a positive OF and a negative DCIP. Normal erythrocyte indices were found for some carriers of α^+-thalassaemia and Hb E, confirming previous findings that these two carriers may have an MCV greater than 80 fl (*21*, *22*). However, this does not matter for α^+-thalassaemia because it is not the target of screening. Carriers of Hb E, however, should not be missed by screenings especially in a south-east Asian community where the prevalence is high.

Interestingly, we obtained excellent results with the DCIP test for Hb E. This simple screening test has 100% sensitivity and 98.7% specificity for Hb E with a positive predictive value of 98.6% and a negative predictive values of 100%. Thus false-

negative tests and false-positive tests are rare. The DCIP test is therefore recommended for screening as an adjunct to the modified OF test.

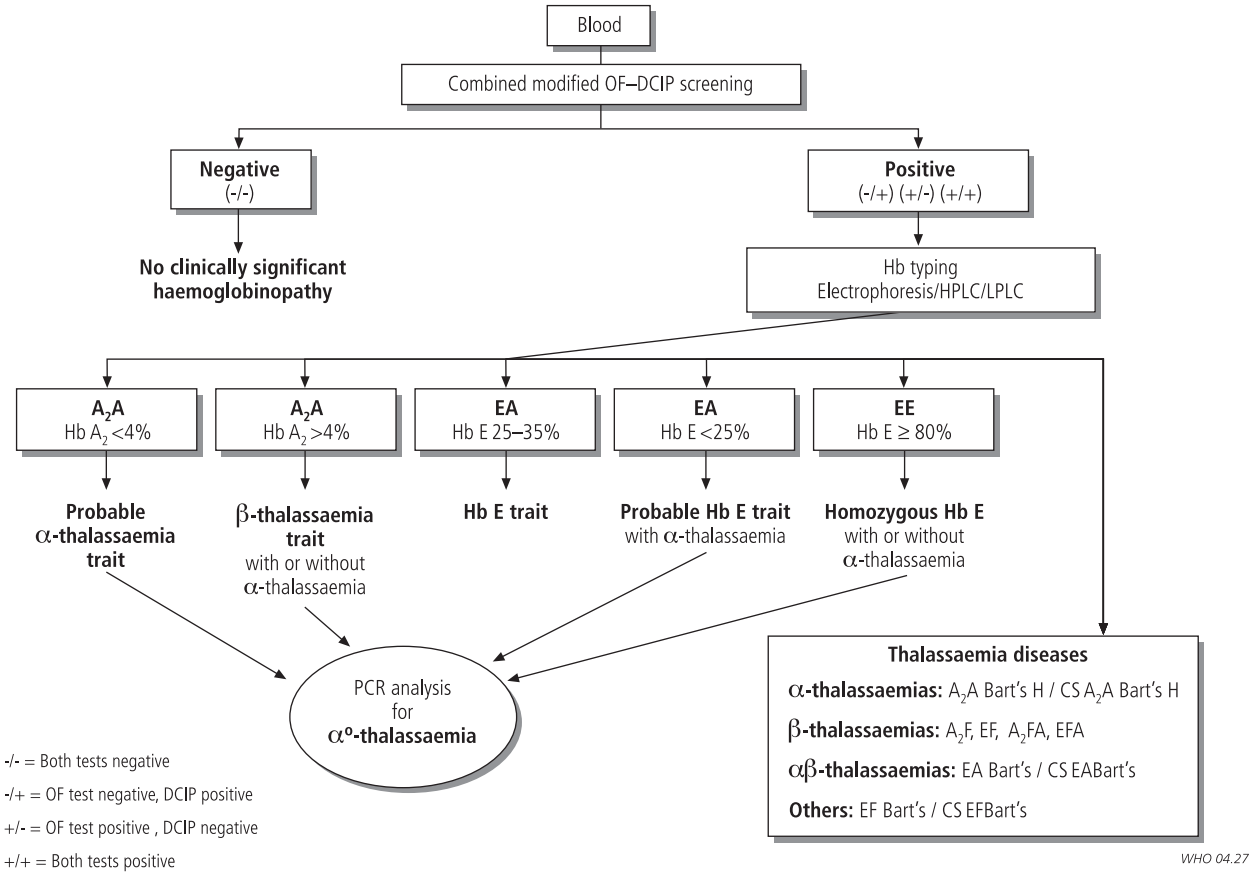
When the data on all 301 participants were analysed (Table 3), we found a sensitivity of 100% and a specificity of 69.8% when using the combined OF–DCIP screening to identify three clinically important groups of carriers (positive predictive value 77.2%; negativ e predictive value 100%). If a standard basal screening procedure (for example, MCV <80 fl and MCH <27 pg) had been used for this population, we would have found 77 negative cases. However, 16 of them were carriers of Hb E. Thus, the standard screening procedure would have had a high false-positive rate and would also miss those participants who are carriers of Hb E. We therefore recommend using the DCIP test in addition to the erythrocyte indices for thalassaemia screening in south-east Asian populations among whom Hb E is prevalent.

When compared with the data obtained using the standard screening procedure, the combined OF–DCIP test identified 104 of 301 participants who had negative results from both tests. None of them was a carrier of α^{o}-thalassaemia, β-thalassaemia

Table 5. **Efficiency of screening using the combined one-tube osmotic fragility test (OF) and modified dichlorophenolindophenol precipitate (DCIP) test for 370 additional participants tested at community hospitals for three forms of thalassaemia: α^{o}-thalassaemia, β-thalassaemia and Hb E.thalassaemia.** See text for information on sensitivity, specificity and positive and negative predictive values

Results of OF/ DCIP screening	Haemoglobin and DNA analysis					
	Hospital A		Hospital B		Hospital C	
	With α^{o}-thalassaemia β-thalassaemia or Hb E	Without α^{o}-thalassaemia β-thalassaemia or Hb E	With α^{o}-thalassaemia β-thalassaemia or Hb E	Without α^{o}-thalassaemia β-thalassaemia or Hb E	With α^{o}-thalassaemia β-thalassaemia or Hb E	Without α^{o}-thalassaemia β-thalassaemia or Hb E
Positive	53	8	51	17	76	18
Negative	0	61	1	51	0	34
Total	**53**	**69**	**52**	**68**	**76**	**52**

Fig. 1. **Proposed screening strategy for carriers of α^{o}–thalassaemia and Hb E using both the modified one-tube osmotic fragility test (OF) and the modified dichlorophenolindophenol precipitation test (DCIP) for preliminary screening**



or Hb E. Thus the combined test has relatively fewer false positives and false negatives. This should, in turn, reduce the need for further Hb and PCR analyses. Of course, as with the other screening protocol, some false positives among people with mild forms of thalassaemia or haemoglobinopathy, as well as iron deficiency, may occur during screening. This does not matter since the major concern of screening is to avoid false negatives. A combination of the OF and DCIP tests is an appropriate preliminary measure that can be used reliably at any small community hospital (Table 4 and Table 5).

The algorithm in Fig. 1 is based on our data and should be applicable to screening programmes in south-east Asia where cost precludes the use of electronic blood-cell counting. The algorithm starts with a preliminary screening using the combined OF–DCIP test (20 µl blood for each test). The kits for these tests are ready to use. The combined test can be done at any community health centre using a finger-prick blood sample. Both tests are simple to use, cheap and give immediate results. Samples that test positive can be sent to a reference laboratory for more detailed investigations using electrophoresis or HPLC and PCR. An additional 10 µl of blood is required for electrophoresis, HPLC or LPLC analysis, and another 10 µl for PCR (*13*). What is most important is that unlike electronic blood-cell counting these confirmatory tests do not require fresh specimens, making transportation of samples more convenient.

Using this combined test for the general population in Thailand (as opposed to the Thai–Khmer population which has a high prevalence of thalassaemia and Hb E) could reduce the workload associated with these investigations by about 50% by eliminating the need for further Hb and PCR analyses or investigation of erythrocyte indices. The proposed screening strategy is, therefore, simple, reliable, cost effective and practical. Using this strategy for a population-based screening programme in a primary health care setting should facilitate prevention and control of thalassaemia and haemoglobinopathies (*24*) in most south-east Asian communities. ■

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Résumé

Stratégie simplifiée pour le dépistage de la thalassémie et de l'hémoglobine E dans des communautés rurales d'Asie du Sud-Est

Objectif Evaluer une stratégie simple de dépistage de la thalassémie et de l'hémoglobine (Hb) E dans le cadre d'un programme de prévention et de lutte axé sur la thalassémie dans des communautés rurales ne disposant que de ressources limitées.

Méthodes Des prélèvements de sang réalisés sur 301 participants thaï-khmers ont été soumis à un dépistage de la thalassémie et de l'HbE au moyen de deux tests combinés, une version modifiée du test de résistance osmotique globulaire en un seul tube (test OF) et une version modifiée du test de précipitation par le dichlorophénolindophénol (test DCIP). Les résultats ont été évalués au moyen d'analyses hématologiques classiques comportant la détermination des indices érythrocytaires, le typage et le dosage de l'hémoglobine et l'analyse par amplification génique (PCR) des gènes de l'α-globine et de la β-globine.

Résultats Les participants ont été répartis en 4 groupes selon les résultats des deux tests. Sur les 301 participants, 104 (34,6 %) avaient des résultats négatifs pour les deux tests, 48 (15,9 %) des résultats positifs pour le test OF mais négatifs pour le test DCIP, 40 (13,3 %) des résultats négatifs pour le test OF mais positifs pour le test DCIP et 109 (36,2 %) des résultats positifs pour les deux tests. Aucun porteur de formes cliniquement importantes de thalassémie (α^0-thalassémie, β-thalassémie) ni d'HbE n'a été trouvé dans le groupe dont les deux tests étaient négatifs. Tous les porteurs d'HbE avaient un test DCIP positif. Les porteurs d'α^+-thalassémie ou d'hémoglobine de Constant Spring pouvaient avoir des résultats positifs ou négatifs pour le test OF mais avaient tous un test DCIP négatif. L'utilisation combinée des deux tests comme méthode de dépistage préliminaire des trois groupes importants de porteurs avait une sensibilité de 100 % et une spécificité de 69,8 %, et une valeur prédictive positive de 77,2 % et négative de 100 %. Une évaluation complémentaire de ce système de dépistage par le personnel local de trois hôpitaux communautaires a donné une sensibilité de 98,1-100 % et une spécificité de 65,4-88,4 %, avec une valeur prédictive positive de 75,0-86,9 % et une valeur prédictive négative de 98,1-100 %.

Conclusion L'association d'un test OF et d'un test DCIP pourrait être utilisée comme alternative efficace au comptage électronique pour le dépistage préliminaire des porteurs d'α^0-thalassémie, de β-thalassémie et d'hémoglobine E. Cette stratégie devrait être utile pour le dépistage en population dans le cadre de programmes de prévention et de lutte menés dans des communautés rurales d'Asie du Sud-Est où les moyens de laboratoire et les ressources financières sont limités.

Resumen

Estrategia simplificada de cribado de la talasemia y la hemoglobina E en comunidades rurales de Asia sudoriental

Objetivo Evaluar una estrategia sencilla de cribado de la talasemia y la hemoglobina (Hb) E en un programa de prevención y control de la talasemia en comunidades rurales con recursos limitados.

Métodos Las muestras de sangre extraídas a 301 participantes de comunidades khmer tailandesas fueron sometidas a cribado para la talasemia y la Hb E mediante una combinación de una prueba

modificada de fragilidad osmótica (FO) y una prueba modificada de precipitación con diclorofenolindofenol (DCIP). Para evaluar los resultados se utilizaron análisis hematológicos ordinarios, incluidos el índice eritrocitario, la tipificación de Hb y la cuantificación y reacción en cadena de la polimerasa (RCP) de los genes de la α-globina y la β-globina.

Resultados Se dividió a los participantes en cuatro grupos según los resultados de las pruebas combinadas. En total, de los 301 participantes, 104 (34,6%) presentaron resultados negativos en ambas pruebas; 48 (15,9%) dieron positivo en la prueba FO pero no en la DCIP; 40 (13,3%) dieron negativo en la FO pero positivo en la DCIP; y 109 (36,2%) dieron positivo en ambas pruebas. No se detectó ningún portador de las formas clínicamente significativas de talasemia (α^0-talasemia, β-talasemia) o de Hb E en el grupo que tuvo resultados negativos en las dos pruebas. Todos los participantes con Hb E fueron positivos en la prueba de DCIP. Los portadores de α^+-thalassaemia o Hb Constant Spring podían dar positivo o negativo en la prueba de FO, pero todos ellos dieron negativo en la prueba DCIP. El uso de ambas pruebas como cribado preliminar de los tres grupos importantes de portadores demostró tener una sensibilidad del 100% y una especificidad del 69,8%. El valor predictivo positivo de la prueba combinada fue del 77,2%, y el valor predictivo negativo del 100%. La posterior evaluación adicional del sistema de cribado por el personal local de tres hospitales comunitarios reveló una sensibilidad del 98,1%–100% y una especificidad del 65,4%–88,4%, con valores predictivos positivos de 75,0–86,9% y valores predictivos negativos de 98,1–100%.

Conclusión Una prueba que combinara la FO y el DCIP podría ser una alternativa eficaz del recuento electrónico de hematíes como cribado preliminar para identificar a los portadores de α^0-talasemia, β-talasemia y Hb E. La estrategia podría ser una valiosa herramienta de cribado poblacional en los programas de prevención y control en las comunidades rurales de Asia sudoriental que cuentan con pocos recursos económicos y medios de laboratorio.

ملخص

استراتيجية مبسطة لتحري الثلاسيمية والهيموغلوبين E في مجتمعات ريفية في جنوب شرق آسيا

الغرض: تقييم استراتيجية بسيطة لتحري الثلاسيمية في مجتمعات ريفية تعاني من محدودية الموارد.

الطريقة: تم أخذ عينات دم من ٣٠١ مشارك من تايلند وكمبوديا لتحري الثلاسيمية والهيموغلوبين E باستخدام اختبار الهشاشة التناضحية المعدَّل الأحادي الأنبوب، والاختبار المعدَّل لترسيب الداي كلوروفينول إندوفينول. وتم تقييم النتائج باستخدام التحاليل الدموية القياسية التي تشمل مناسب كريات الدم الحمراء، وتحديد نمط الهيموغلوبين وتقديره كميا، وتحليل جينات ألفا غلوبين وبيتا غلوبين عن طريق التفاعل السلسلي للبوليميراز.

الموجودات: تم تقسيم المشاركين إلى أربع مجموعات وفقاً لنتائج الاختبارات المشتركة. وتبين أن ١٠٤ مشاركين من جملة المشاركين البالغ عددهم ٣٠١ مشارك (أي بنسبة ٣٤,٦٪) حصلوا على نتائج سلبية في الاختبارين؛ وكان ٤٨ مشاركاً (أي بنسبة ١٥,٩٪) إيجابيين لاختبار الهشاشة التناضحية وليس لاختبار ترسيب الداي كلوروفينول إندوفينول؛ وكان ٤٠ من المشاركين (١٣,٣٪) سلبيين لاختبار الهشاشة التناضحية ولكنهم إيجابيون لاختبار ترسيب الداي كلوروفينول اندوفينول؛ وكان ١٠٩ من المشاركين (٣٦,٢٪) إيجابيين للاختبارين. و لم يوجد بين مجموعة المشاركين السلبيين للاختبارين أي شخص يحمل أنماطا من الثلاسيمية ذات الأهمية السريرية (مثل ألفا ثلاسيمية α^0 أو بيتا ثلاسيمية β) أو يحمل الهيموغلوبين E. ولوحظ أيضاً أن جميع المشاركين الذين ثبت وجود الهيموغلوبين E لديهم كانوا إيجابيين لاختبارات ترسيب الداي كلوروفينول إندوفينول. أما الحاملون للثلاسيمية α^+ أو الهيموغلوبين اللولبي الثابت فقد يحصلون على نتائج سلبية أو إيجابية لاختبار الهشاشة التناضحية، ولكنهم جميعا سلبيون لاختبار ترسيب الداي كلوروفينول إندوفينول. وقد أسفر استخدام الاختبارين في التحري التمهيدي للمجموعات الرئيسية الثلاث عن حساسية ١٠٠٪ و نوعية ٦٩,٨٪ . وكانت القيمة التنبؤية الإيجابية للاختبار المشترك ٧٧,٢٪. أما القيمة التنبؤية السلبية فكانت ١٠٠٪. وتبين من التقييم الإضافي لنظام التحري ، الذي أجراه العاملون المحليون في ثلاثة مستشفيات مجتمعية، أن الحساسية بلغت ٩٨,١٪ إلى ١٠٠٪ وأن النوعية بلغت ٦٥,٤٪ إلى ٨٨,٤٪، وأن القيم التنبؤية الإيجابية بلغت ٧٥٪ إلى ٨٦,٩٪، وأن القيم التنبؤية السلبية بلغت ٩٨,١ ٪ إلى ١٠٠٪.

الحصيلة: من الممكن إجراء الاختبار المشترك الذي يشمل اختبار الهشاشة التناضحية واختبار ترسيب الداي كلوروفينول إندوفينول كوسيلة فعالة للتحري المبدئي لكشف حاملي الألفا ثلاسيمية ، والبيتا ثلاسيمية ، والهيموغلوبين E، بدلاً من عدّ كريات الدم. وينبغي أن تثبت فائدة هذه الاستراتيجية في تحري السكان في إطار برامج الوقاية والمكافحة في المجتمعات الريفية في جنوب شرق آسيا التي تعاني من محدودية المختبرات والموارد الاقتصادية.

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