

Assessment of thalassemia screening program at peripheral health care facilities

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บทคัดย่อ

ประเทศไทยได้มีการดำเนินงานตรวจคัดกรองธาลัสซีเมียมาเป็นเวลานานกว่าหนึ่งทศวรรษ การศึกษานี้มีวัตถุประสงค์เพื่อประเมินการดำเนินงานและความถูกต้องของโปรแกรมการตรวจคัดกรองในสถานบริการสุขภาพระดับชุมชน โดยรวบรวมตัวอย่างเลือดที่เหลือของผู้มารับบริการตรวจคัดกรองธาลัสซีเมียพร้อมผลตรวจทั้งบวกและลบ จากโรงพยาบาลชุมชน ในภาคตะวันออกเฉียงเหนือ 11 แห่ง รวมทั้งสิ้น 1,200 ตัวอย่าง นำมาตรวจวินิจฉัยธาลัสซีเมียที่ ศูนย์วิจัยและพัฒนาการตรวจวินิจฉัยทางห้องปฏิบัติการทางการแพทย์ (ศวป.) คณะเทคนิคการแพทย์ มหาวิทยาลัยขอนแก่น จากผลการการตรวจวิเคราะห์ชนิดฮีโมโกลบินและดีเอ็นเอ พบความชุก α^0 -thalassemia (α^0 -thal) ร้อยละ 5.6, β -thalassemia ร้อยละ 1.0 และ hemoglobin E (Hb E) ร้อยละ 41.4 แต่ละพื้นที่มีอัตราการตรวจพบพาหะ α^0 -thal แตกต่างกันมาก ตั้งแต่ร้อยละ 1.9 ถึงร้อยละ 16.0 ในขณะที่อัตราการตรวจพบ β -thal ไม่แตกต่างกัน และพบผลตรวจคัดกรองที่เป็นบวก 574 ตัวอย่าง จากจำนวน 1,200 ตัวอย่าง (ร้อยละ 47.8) เมื่อประเมินการดำเนินงานและความถูกต้องของการตรวจคัดกรองของแต่ละโรงพยาบาล พบว่า 3 โรงพยาบาล มีผลตรวจคัดกรองที่ไม่น่าพอใจนัก โดยพบอัตราผลลบปลอมมากถึงร้อยละ 10 และผลบวกปลอมมากกว่าร้อยละ 30 ผลการประเมินหลังให้เข้ารับการอบรม พบว่า ทั้งสามโรงพยาบาลมีผลการดำเนินงานตรวจคัดกรองที่พัฒนาขึ้น โดยมีอัตราผลลบปลอมน้อยกว่าร้อยละ 10 ผลการศึกษา บ่งชี้ว่า ควรมีการจัดตั้งระบบการทดสอบความชำนาญและการจัดอบรมอย่างสม่ำเสมอ เพื่อลดอัตราผลลบปลอมและกำกับติดตามการดำเนินงานตรวจคัดกรองธาลัสซีเมียของโรงพยาบาลชุมชน

คำสำคัญ: การตรวจคัดกรองธาลัสซีเมีย, การทดสอบ OF, การทดสอบ DCIP

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Assessment of thalassemia screening program at peripheral health care facilities

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Abstract

In Thailand, screening program for thalassemia has been implemented for more than a decade. In order to examine the operation and accuracy of thalassemia screening program at peripheral health care facilities, 1,200 left-over blood samples derived from subjects with both negative and positive screening results from 11 community hospitals in northeast Thailand were collected and sent to the Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, Khon Kaen University, for further determinations of thalassaemia and hemoglobinopathies. Based on hemoglobin and DNA analyses, the overall prevalence of α^0 -thalassemia (α^0 -thal) was 5.6 %, of β -thalassemia (β -thal) 1.0 % and of hemoglobin E (Hb E) 41.4 %. While the proportion of β -thal and Hb E in each setting was similar, the rate of α^0 -thal varied from 1.9 % to 16.0 % between the locations. Of 1,200 samples, 574 (47.8 %) were positive for thalassemia screening. Assessing the operation and accuracy of thalassemia screening revealed unsatisfactory results in 3 laboratories with FN > 10 % and/or FP > 30 %. After conducting the training program, their performance was substantially improved with a FN rate of less than 10.0 %. The findings indicate that proficiency testing and regular training programs are needed to decrease false negative results and to monitor regularly the screening performance of the community hospitals.

Keywords: Thalassemia screening, OF-test, DCIP-test

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Introduction

Thalassemia and hemoglobinopathies are amongst the major public health problems worldwide⁽¹⁾. In Thailand, prevention and control program for thalassemia has been implemented for more than a decade. The three prime targets are homozygous α^0 -thalassemia (α^0 -thal), homozygous β -thalassemia (β -thal) and hemoglobin E (Hb E)- β -thal diseases^(2,3). All pregnant women visiting a hospital or other medical centre for antenatal care (ANC) the first time with a gestational age of less than 16 weeks are asked to participate in the screening program⁽⁴⁾. The participation is voluntary. A combination of basic turbidity tests, the osmotic fragility (OF) test and the dichlorophenol-indophenol (DCIP) precipitation test is recommended as a primary screening tool^(5,6). Alternative to the OF-test, the mean corpuscular volume (MCV) of red blood cell with a cutoff value of 80 femtolitre (fl) may be used. The positive blood samples are then sent to a reference centre to confirm or exclude the diagnosis. Individuals with negative screening results are considered as not having any form of thalassemia or having a form of the disease not being clinically significant and no further investigation is required. Based on this strategy, the accuracy of the screening tests is a key issue hence a high rate of false negative (FN) will undermine the efforts for prevention and control of the diseases since a substantive number of diseased person will go unrecognized. On the other hand, a high false positive (FP) rate will result in a high workload for the reference centers and budget spent uselessly.

Laboratory methods for thalassemia screening are relatively simple and have been proven to be an effective tool by many investigators⁽⁷⁻⁹⁾. However, FN results had been found often by the laboratory of the authors and did lead to a query concerning the accuracy of the screening procedures conducted at peripheral health care facilities where human resources

are limited. In this study, we implemented a proficiency testing program and evaluated the FN and FP rates resulting from thalassemia screening conducted at community hospitals in northeastern Thailand, an area where thalassemia is prevalent.

Materials and Methods

A cross sectional study investigating the performance of thalassemia screening was launched in 11 community hospitals located in different regions of northeastern Thailand. Specimens used for this study had been left-over blood samples obtained from pregnant women and their husbands attending ANC services. Data recorded for the thalassemia screening could not be traced back to any individual taken part in the program. The study protocol was approved by the ethical committees of the Khon Kaen University, Khon Kaen, Thailand (HE510139).

The director of each hospital was invited to participate and asked for permission to conduct the study. Laboratory staffs were informed about the project and asked for their corporation. The study protocol included implementation of proficiency testing program and evaluation of FN and FP rates as well as problem analysis and conducting professional training to those laboratories with poor performance. Activities performed were as follows; firstly, laboratory staffs were asked to perform thalassemia screening according to the regular methods used in their laboratory. Blood samples derived from women with both negative and positive results, were sent to the Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, Khon Kaen University, for further determinations of thalassemia and hemoglobinopathies. The FN and FP rates were calculated to determine the performance of each laboratory. FN rate < 5 % and FP rate < 30% were used to certify the good performance. Laboratories with FN rate 5-10 % were considered acceptable.

Laboratories with FN > 10 % and/or FP > 30 % were included to analyze the problem and implement the training program. After training, each laboratory was asked to send blood samples again to the CMDL to evaluate the performance of each individual laboratory a second time.

Screening strategies

According to the national guideline (Figure 1), most laboratories either used a combined OF / DCIP or MCV / DCIP test^(5,6) for screening. The OF and DCIP tests are commercially available. In brief, OF test was done by adding 20 microlitre (µl) of fresh

blood sample into 2 millilitre (ml) of hypotonic buffer saline provided by the manufacturer. After mixing gently, the mixture was left at room temperature for at least 15 minutes. For DCIP test, 20 µl of blood sample was added into 2 ml of the DCIP reagent. After mixing, the mixture was incubated at 37°C for 15 minutes; and then, 20 µl of additional solution for stopping the reaction was added. The interpretation of both tests was made according to the turbidity of each reaction tube. The turbidity visible by naked-eye was considered positive. In case of MCV screening, the MCV < 80 fl was considered positive.

1. Peripheral health care facilities

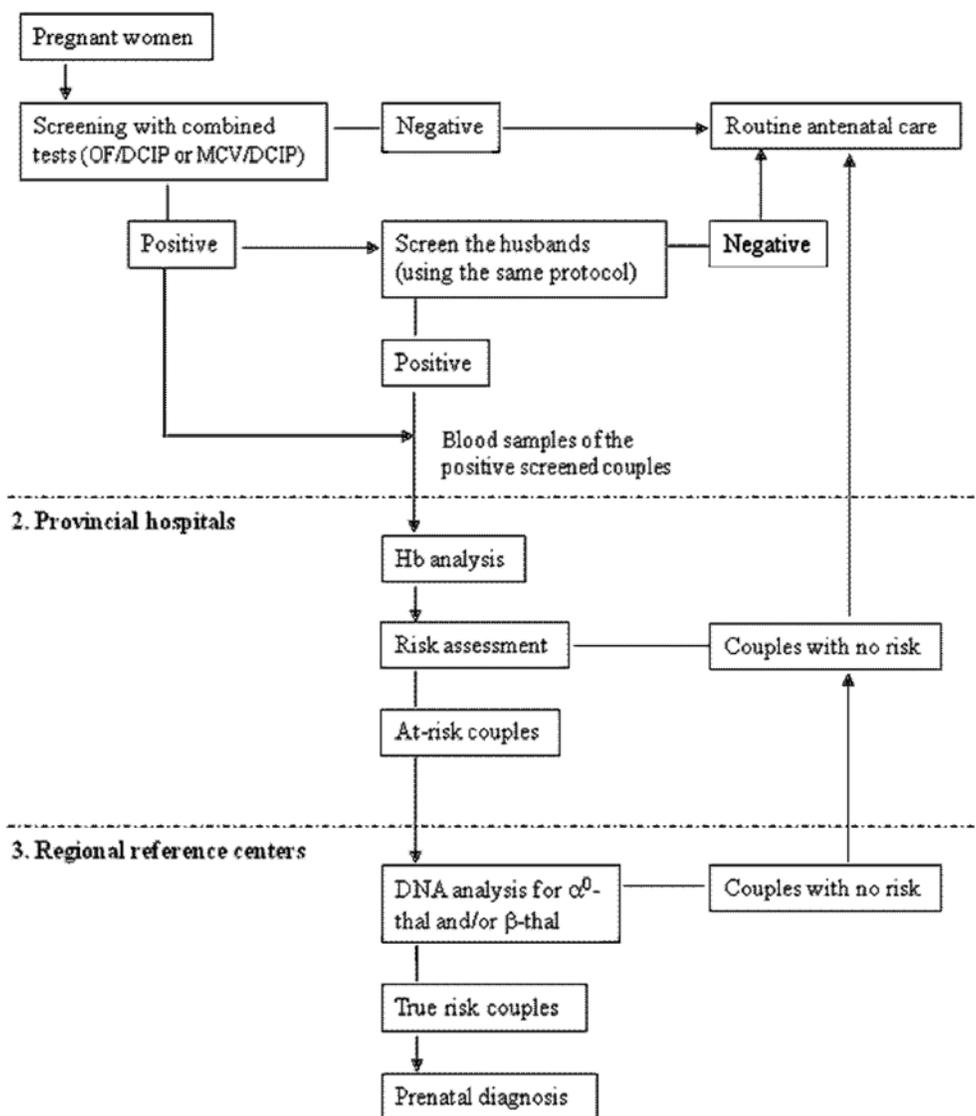


Figure 1 Flow diagram of a national guideline for thalassemia screening (adapted from reference No.5)

Laboratory investigation of the reference centre

All blood samples with positive and negative results were investigated further for β -thal and Hb E using an automated hemoglobin analyzer, either the high performance liquid chromatography (HPLC Variant Hemoglobin Testing System; Bio-Rad, USA.) or the capillary zone electrophoresis (Capillary; Sebia, France). Samples with normal Hb-type (A_2A) and Hb $A_2 \geq 3.5\%$ were diagnosed as β -thal carriers. α^0 -Thal (SEA & THAI deletions) was identified using the polymerase chain reaction (PCR) ⁽¹⁰⁾.

Training program

Training program on thalassemia screening took place at the hospitals. It was provided to laboratory staffs of those hospitals with a poor performance. The training focused on the principle, procedure and interpretation as well as limitation of the OF/DCIP tests. A poster of the standard operation procedure (SOP) of the OF and DCIP tests was created as an educational material and distributed to all community hospitals participated.

Data analysis

To determine the performance of thalassemia screening, FN and FP rates were calculated. Tests con-

ducted by the local laboratory staff using the combined methods of OF/DCIP or MCV/DCIP and found to be negative but were actually carriers of α^0 -thal, β -thal or Hb E as found by the reference centre in using the methods as described above, were considered as false negative. The cases identified by the screening tests as positive but not being confirmed with the tests of the reference centre were considered to be false positive.

Results

Of the 11 community hospitals, 9 laboratories used a combined OF/DCIP for thalassemia screening. A combined MCV/DCIP was used in one hospital. The other used a 2 step thalassemia screening; i.e. firstly screened with the MCV, the DCIP test was performed only in cases with negative MCV screening.

A total of 1,200 blood samples screened for thalassemia from 11 community hospitals were investigated. Of these, 574 (47.8 %) were positive for thalassemia screening. Based on the 1,200 samples as denominator, the rate of α^0 -thal was 5.6 %, of β -thal 1.0 % and of Hb E 41.4 %. While the proportion of β -thal and Hb E from each hospital was similar, the rate of α^0 -thal varied from 1.9 % to 16.0 % between the locations (Table 1). These results confirm the high prevalence of Hb E and α^0 -thalassemia in the region.

Table 1 Proportions of α^0 -thalassemia, β -thalassemia and Hb E in northeastern Thai-subjects attending ANC in 11 community hospitals

Hospital No.	N	Proportions (%)		
		α^0 -thal	β -thal	Hb E
1	100	7.0	1.0	41.0
2	130	7.6	0.76	38.5
3	122	2.5	0.82	35.2
4	101	6.9	1.0	48.5
5	103	1.9	0.97	48.5
6	100	8.0	1.0	44
7	98	4.1	2.0	41.8
8	103	2.9	0.97	48.5
9	132	3.0	1.5	47.0
10	100	16.0	1.0	34.0
11	111	2.7	0	29.7
Total	1,200	5.6	1.0	41.4

In average 7.2 % were false negative and 7.7 % false positive. FN and FP rates by hospitals are given in **Table 2**. The FN and FP rates of OF for screening of α^0 -thal and β -thal and DCIP for screening of Hb E were calculated separately. It was found that 8 out of 11 hospitals had acceptable performance with FN < 5 % and FP < 30 %. These 8 hospitals were certified. The other three performed poorly with very high FN rates of 18.0-60.9 % for a combined OF/DCIP test,

22.2 to 66.7 % for the OF-test and 50 to 78.1 % for the DCIP test. The FP rate varied considerably from 5.8 to 30.2 % for OF/DCIP, 15.2 to 43.0 % for OF test and 0 to 25.8 % for the DCIP test.

In order to improve the performance of screening, a training program was organized at the 3 hospitals with a poor performance. Re-evaluation of the FN rate indicated a substantially improved performance resulting in FN rates of less than 10.0 % (**Table 3**).

Table 2 False negative rate (FN) and false positive rates (FP) of thalassemia screening of 11 community hospitals

Hospital No.	N	FN (%)			FP (%)		
		OF/DCIP	OF	DCIP	OF/DCIP	OF	DCIP
1	103	1.9	0	4.0	16	43.0	0
2	122	2.2	0	4.7	21.1	31.4	3.8
3	100	2.3	0	2.4	8.9	25.0	5.1
4	111	3.1	0	6.1	19.0	35.2	0
5	130	4.3	na	na	9.2	na	na
6	100	4.3	5.9	14.7	30.2	25.3	25.8
7	103	5.6	0	6.0	12.2	28.3	1.9
8	101	8.3	0	13.0	8.3	24.7	6.0
9	100	18	22.2	50	14	29.7	8.9
10	132	51.5	33.3	72.1	9.4	21.4	1.4
11	98	60.9	66.7	78.1	5.8	15.2	1.8

na: not applicable

Table 3 Comparison of false negative rate before and after implementation of training program in 3 community hospitals

Hospital No.	OF/DCIP		OF		DCIP	
	Before	After	Before	After	Before	After
1	18	0	22.2	0	50	4.3
2	60.9	2.0	66.7	0	78.1	7.1
3	51.5	6.7	33.3	0	72.1	9.1

Discussion

The screening program for thalassemia in Thailand generally includes three steps; i.e. antenatal screening, genetic counseling and prenatal diagnosis for at-risk couples. For α - and β -thalassemia screening, the approach using MCV and/or MCH has been recommended in European countries^(11,12). This approach could not be used in Thailand and other Southeast Asian countries due to a high prevalence of Hb E

(Table 1) going along with varying MCV value⁽⁵⁾. Therefore, the DCIP precipitation test in combination with MCV had been introduced to identify the three forms of thalassemia carriers, α^0 -thal, β -thal and Hb E. However, due to budget limitation the electronic blood cell counter for MCV measurement is not available all over the country, therefore, the OF/DCIP is an alternatively effective option for thalassemia screening and has been adopted as a national guideline. Based on

the guideline, pregnant women with negative screening results are considered not being carriers of the diseases or having a form of non-clinically significant thalassemia and no further actions are required (Figure 1). The husbands of positive-screened women are invited to be screened for thalassemia as well using the same screening methods. The positive couples (i.e., thalassemia screening positive for both husband and wife) are, then, investigated further to make certain that the diagnosis is true. For the screening program it is of immense importance to keep false negative results as low as possible.

It should be noticed that 8 laboratories out of 11 ones produced sufficient screening results. So it can be assumed that screening results for thalassemia throughout northeastern Thailand are well acceptable. However further improvement would be of great benefit as well. As a matter of fact, thalassemia screening using the OF/DCIP and MCV/DCIP approach has been proven to be effective in expert hands⁽⁵⁻⁸⁾. However, this may not be true in a situation when the local health staff is not well trained. As shown in Table 2, although the performance of 8 out of 11 hospitals were acceptable with a FN rate less than 5 %, 3 hospitals showed a poor performance with high FN rates (hospital No.9, 10 and 11, Table 2).

The main reasons for the high FN and FP rates was due to the difficulties in interpreting the results of the OF/DCIP test being used as screening test. Positive and negative results are based on the turbidity of test reaction which could vary from sample to sample depending mainly on the thalassemia type and the hemoglobin concentration in the samples being tested. Hence laboratory staffs need experiences and skills to judge whether the test is positive or negative. It was found that for 2 out of the 3 hospitals the main problem was to read the results properly from the turbidity of the test reaction. The other hospital used not well calibrated equipment, which referred in particular

to the temperature used for the DCIP incubation. It is well recognized that the DCIP test needs careful training⁽¹³⁾. The training therefore focused on that test and the results given in Table 3 are indicative of the training success.

Additional consideration have to be given to the OF test. It should be noted that the FP rates found in this study vary considerably (Table 2) also due to the difficulties with the OF test. It has been shown that the specificity of the test is not as high as for the DCIP test in an area where the prevalence of thalassemia is high and heterogeneous. Other forms of a-thalassemia as well as Hb E could lead to positive OF screening result^(5,6). In addition, iron deficiency which is also prevalent in the region might be the cause of FP results. Therefore, a high FP rate of 25 to 30% is acceptable. However, it is shown that the FP rate of some hospitals is even higher than 30%.

As a matter of fact, it has been shown previously in our pilot study that the performance of thalassemia screening at peripheral health care facilities may require particular attention⁽¹⁴⁾. In this study, we implement the proficiency testing program in particular to the northeastern region of Thailand. The screening results did confirm a high false negative rate in some settings. In concordance with this study, a great improvement of screening performance was obtained after introducing training program and the standard operation procedure as well as the quality control samples to the laboratory staffs⁽¹⁴⁻¹⁷⁾. The results of the these studies indicate that although the tests are simple and easy, laboratory staffs need to be well trained and indicate an urgent need of regular training and proficiency testing as well as internal quality control system to monitor the screening performance of community hospitals throughout the country.

It should be noted that only positive screened couples are subject to further investigation but with a high FN rate a number of couples with the disease

remain undetected and not cared for. The staffs of the hospitals with insufficient performances were surprised when faced with the high unexpected FN rate. It was found out, that they have never attended the training program for thalassemia screening. In addition, no quality control system had been introduced. This is probably due to limitation of the tests that require fresh specimen. Interestingly, although these two tests have been developed for quite a long time and have already provided as reagent kits, up till now, no quality control products are available. The kits have also been used in the Lao People's Democratic Republic ^(18,19). It is, therefore, challenged to scientist to develop such products for these simple screening tests to reduce the error in practicing of laboratory staffs.

Overall the prevention and control program of thalassemia in Thailand has progressed considerably. Thalassemia screening is being done almost in all community hospitals. The results of this study provide an important lesson for not only Thailand but also other countries that already launched similar programs for prevention and control of the disease. It should be realized that false negative thalassemia screening could affect not only the achievement of the prevention and control program but also will pose a psychological trauma to pregnant women if they deliver babies having a severe form of thalassemia. False negative results also might create legal problems to the health staff and possibly the policy makers.

Though, the finding of the study is pointed mainly to the errors of laboratory screening, it should be emphasized that the consequence of errors affects directly to not only the patients but also health staffs and obstetricians involved in the genetic counseling and prenatal diagnosis. Monitoring and proficiency testing therefore is essential to initiate awareness of the health staff.

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