

PILOT SCREENING PROGRAM FOR THALASSEMIA IN A COUNTRY WITH LIMITED RESOURCES: A COLLABORATION MODEL BETWEEN CLOSE NEIGHBORING COUNTRIES

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Abstract. Screening for thalassemia carriers should not only be conducted in middle-income countries but also can be possible in low-middle income countries, through cooperation of experienced professionals from middle income countries. We describe a collaborating model between two close neighboring countries in establishing such a screening program for thalassemia. After training and setting up of facilities, a total of 152 out of 187 hospital staff were screened as a pilot activity to encourage community participation. Referring system for sending blood samples to a reference center in Thailand was also established. Among 152 health staff, 12.5% α^0 -thal, 2% β -thal and 13% Hb E carriers were found. Applying thalassemia screening to 411 pregnant women and 71 spouses, 5 couples at risk of bearing a child of thalassemia disease were identified. The thalassemia screening program has a sensitivity of 99.5%, specificity of 77%, positive predictive value of 73%, and negative predictive value of 99.5%. Thus, it is possible to operate a thalassemia screening program with acceptable performance in a low-middle income country (Lao People's Democratic Republic) with the cooperation of a referral center located within close proximity in a middle income country (Thailand).

Keywords: thalassemia, hemoglobinopathy, screening program, Lao PDR

INTRODUCTION

The most common genetic diseases causing serious burden for healthcare

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systems include thalassemias and hemoglobinopathies (William and Weatherall, 2012). Individuals with severe thalassemia diseases suffer lifelong from serious health problems, and their families suffer not only mentally but also economically due to their need of lifelong medical attention. In countries where thalassemia and hemoglobinopathies are common, preven-

tive measures are needed. Individuals who are carriers of thalassemia should be informed about the risk when they marry or plan to bear children.

Carriers of thalassemia (autosomal inherited) usually have no clinical symptoms, and can be detected only through a screening program. A number of countries have initiated screening procedures aiming to decrease the incidence of severe forms of thalassemia (Fucharoen and Weatherall, 2016). In a number of countries, *viz*, Thailand, Malaysia, China and India, such screening initiatives are associated with antenatal care (ANC) services or family planning programs encouraging women in the early stage of pregnancy and their partners to be tested for thalassemia (Fucharoen and Winichagoon, 1992; Ainoon and Cheong, 1994; Indaratna, 1997; Jaovisidha *et al*, 2000; Liao *et al*, 2005; Choudhuri *et al*, 2015). In a situation where there is a high risk of conceiving a child with a severe thalassemia, prenatal diagnosis (PND) of the fetus is suggested, followed by in-depth counseling regarding possible options (WHO, 1994; Weatherall, 2010; William and Weatherall, 2012). In Thailand, this type of program was established with focus on reducing the number of births of 3 major thalassemia disease, namely, Hb Bart's hydrops fetalis (the most severe form that results in no live birth), homozygous β -thalassemia, and Hb E/ β -thalassemia, and providing best care and treatment for those with the diseases and their mothers (Fucharoen and Winichagoon, 1992; Winichagoon *et al*, 2002).

Joint investigations between neighboring Lao PDR and Thailand revealed a high prevalence of thalassemia carriers among pregnant women in Vientiane: 8-12% α^0 -thalassemia (α^0 -thal), 2-3% β -thalassemia (β -thal) and 25-30% Hb E

(Savongsy *et al*, 2008; Tritipsombut *et al*, 2012). This indicates that thalassemia syndromes constitute a serious health burden in the country, and prevention and control programs should be established. This could be initiated by technical cooperation with Thailand, a high-middle income country. A suitable setting for such an initiative is available because of the location of the Khon Kaen University (KKU) in Northeast Thailand, in close vicinity of Vientiane's academic as well as health institutions. Cooperation between both countries, especially at the technical level, has been on-going for quite a long period (Chongsuphajaisiddhi and Salazar, 1998).

In order to explore the feasibility of implementing a screening program for thalassemia in Lao PDR, a pilot collaborating project between the two countries has been established (Wongprachum *et al*, 2016). In this communication, we describe a collaborating model for screening thalassemia in a country with limited resources, with the aim of evaluating the capability of a hospital and the staff in the vicinity of Vientiane to set up and carry out a thalassemia screening program.

MATERIALS AND METHODS

Site location

A pilot project of thalassemia screening was initiated at a Vientiane provincial hospital, the Maria Teresa Hospital. The hospital is located approximately 70 km from Vientiane and 260 km from Khon Kaen Province, Thailand (Fig 1). The project was conducted in collaboration with the Center for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, KKU, Khon Kaen.

The study protocol was approved by the Ethics Committee of Khon Kaen Uni-

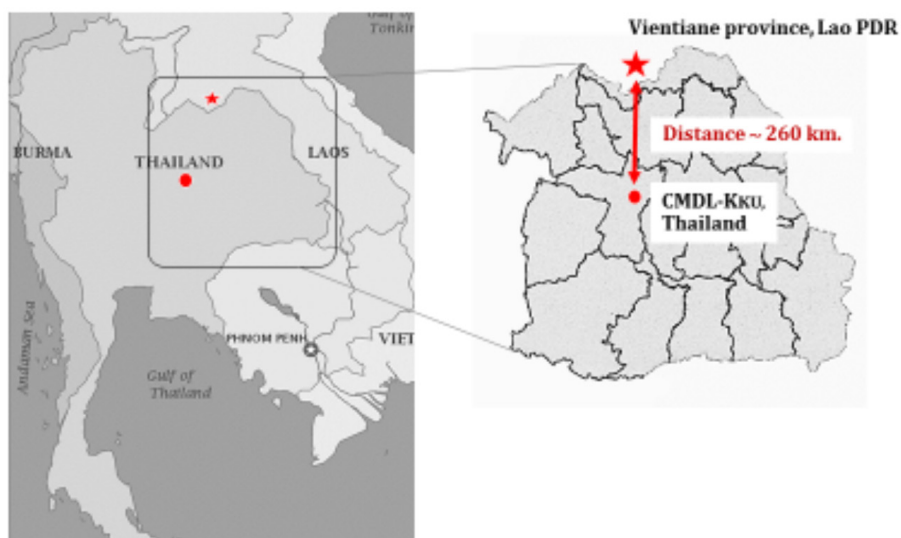


Fig 1–Map showing the locations of local setting in Lao PDR and the Center for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University (CMDL-KKU), Khon Kaen, Thailand.

versity (HE551414) as well as by the provincial health authorities of Vientiane Province (VHO2492). All participants provided written consent prior to being enrolled in the program.

Project phases

Phase 1: Establishment of cooperation, facilities and manpower for thalassemia screening at Maria Teresa Hospital. Meetings between the provincial and district health authorities and experts from KKU were organized several times before implementing the program. Key issues of discussion included establishment of (i) facilities for laboratory screening, (ii) genetic counseling protocols and (iii) system for transporting blood samples to a reference center as well as for co-operating among relevant health workers and members of the local community. One competent Maria Teresa Hospital staff with substantial knowledge of thalassemia was selected as the project coordinator.

Training programs including basic knowledge concerning thalassemia and

screening methods for thalassemia were provided to the relevant health staff. Doctors, nurses and laboratory staff, who were assigned to be in charge of screening and counseling, were invited to Khon Kaen University for intensive training on technical and genetic counseling aspects. Materials essential for educating and communicating with the public, such as pamphlet, teaching aids and flipchart, were developed in the Lao language. An on-site workshop on thalassemia screening was organized where experienced staff members from KKU stayed at Maria Teresa Hospital for 3 weeks to assist in setting up the laboratory facilities. In order to motivate participation of the local health workers, blood testing for thalassemia was offered to all staff of Maria Teresa Hospital.

Phase 2: Conducting thalassemia screening of pregnant women at Maria Teresa Hospital. Antenatal screening for thalassemia used at Khon Kaen University was applied to pregnant women attending an

THALASSEMIA SCREENING IN LAO PDR

Local setting		Reference center
Antenatal screening for thalassemia Blood testing using either OF/DCIP or MCV DCIP		
Providing primary counseling to the positive-screened couple Referring positive-screened blood sample to the reference center		
	Logistic system (Public transport) →	Laboratory diagnosis of thalassemia (Hb and DNA analyses) Reporting & interpreting the results of thalassemia as well as assessing the risk
Intensive counseling to the at-risk couple (inform the choice of PND)	← Electronic mailing system	
	Logistic system (Public transport) →	Providing PND* & DNA analysis for the fetus Reporting & interpreting the results of the fetus
Providing the choice of termination of pregnancy (in case of confirmed risk)	← Electronic mailing system	

*PND can be performed in any other hospital that has obstetrician.

Fig 2–Diagram depicting the work flow between local setting (Maria Teresa Hospital, Vientiane, Lao PDR) and reference center (the Center for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand) in setting up the screening program for thalassemia.

antenatal care (ANC) service for the first time at Maria Teresa Hospital. Only pregnant women with a gestational age of < 16 weeks were enrolled. Essential knowledge of thalassemia disease was explained to all pregnant women voluntarily enrolled in the project. Blood samples were taken and sent to the laboratory department to screen for thalassemia by measuring mean corpuscular volume (MCV) in combination with dichlorophenolindophenol precipitation (DCIP) test (Fucharoen *et al*, 2004; Sanchaisuriya *et al*, 2005). Primary counseling was provided to women screened positive (either MCV < 78 fl or positive DCIP test, or both) (Chaitriphop *et al*, 2016) and were asked to invite their partners to be screened for thalassemia

as well. All women screened negative were also informed about the results and continued routine ANC service. All blood samples (both screened positive and negative) were kept at 2-6°C and sent on ice to CMDL, Khon Kaen University once a week. Hb and DNA analyses were performed to diagnose thalassemia as previously described (Yamsri *et al*, 2011; Chaibunruang *et al*, 2013). Accuracy of thalassemia screening was validated by comparing the screening results with the diagnostic results. The project coordinator was informed of the results (via e-mail). To ensure confidentiality, all specimens were coded and only pertinent staff of Maria Teresa Hospital staff knew the names of the blood donors. Genetic counseling was

Table 1
Thalassemia traits and diseases, Hb variant and Hb levels among 152 staff of Maria Teresa Hospital, Vientiane, Lao PDR.

Category	Number (%)	Hb level (g/dl)
Hb E trait	36 (24)	11.4±1.3 (9.7-15.2) ^a
α^0 -Thal trait	14 (9)	10.0±1.0 (8.8-12.0) ^a
Hb E trait/ α^0 -thal trait	2 (1)	10.0, 11.5
β -Thal trait	2 (1)	9.5, NA
Homozygous Hb E	1 (0.5)	8.4
Hb H disease	2 (1)	7.1, 9.5
Hb E/ β -thal disease	1 (0.5)	6.5
Normal and α^+ -thal trait	94 (62)	11.7±1.3 (8.4-14.6) ^a

^aMean ± standard deviation (range). NA, not available.

provided to at-risk couples by trained counselors, either a doctor or a nurse, who explained the risk of the baby being born with a severe form of thalassemia and advised the option of a prenatal diagnosis (PND) to be performed at Srinagarind Hospital, Khon Kaen, Thailand. The work flow is shown in Fig 2.

Statistical analysis

Sensitivity, specificity, positive predictive value (the probability that positive-screened individuals truly have the disease), and negative predictive value (the probability that negative-screened individuals truly do not have the disease) were calculated and considered as key indicators determining the feasibility of implementing the screening program in this setting. The target carriers included the three clinically significant thalassemias, namely, α^0 -thal, β -thal and Hb E.

RESULTS

Following training and establishment of facilities for thalassemia screening, 152/187 (81%) hospital staff were screened as a pilot trial, revealing a prevalence of 9%, 1% and 24% for α^0 -thal, β -thal and

Hb E trait, respectively (Table 1). There were two cases of Hb H disease (α^0 -thal/ α^+ -thal) and one of Hb E/ β -thal disease. In addition there were two cases of Hb E trait with co-inheritance of α^0 -thal trait and there was one case of homozygous Hb E. The remaining 94 subjects were normal or carriers of α^+ -thal (the latter not included the diagnostic assays). Hb levels of all carriers were within normal range, but those of thalassemic disease or homozygous Hb E were below normal range.

A total of 411 pregnant women and 71 spouses participated in the pilot project. Sensitivity, specificity, positive predictive value, and negative predictive value of the screening test conducted on these participants was 99.5%, 77%, 73%, and 99.5%, respectively (Table 2). Among the participating couples, 40 couples (56%) were screened positive, with three mothers at risk of bearing a child with homozygous α^0 -thal (Hb Bart's hydrops fetalis) and two for Hb E/ β^0 -thal disease.

DISCUSSION

In many low-middle income countries, health care services for genetic

Table 2
Thalassemia screening and diagnosis of 482 participants at Maria Teresa Hospital,
Vientiane, Lao PDR.

Screening test	Diagnosis		Total
	Target carrier ^a	Non-target case ^b	
Positive	184	69	253
Negative	1	228	229
Total	185	297	482

^a α^0 -Thal, β -thal and Hb E. ^bNormal and α^+ -thal carrier.

diseases do not exist because of the lack of basic epidemiological information, infrastructure, human resources as well as recognition of the problem from authorities (Tekola-Ayele and Rotimi, 2015). In Lao PDR, the law on health care gives the right to provincial and district health authorities to provide health services and implement necessary public health measures (WHO, 2011). The strong evidences of a high prevalence of thalassemia carriers (Savongsy *et al*, 2008; Tritipsombut *et al*, 2012), the frequent occurrences of Hb Bart's hydrops fetalis and severe thalassemia syndromes observed within the local setting have led provincial and district health authorities to initiate a pilot screening program for thalassemia carriers at a Vientiane provincial hospital. A combined MCV/DCIP test was chosen to be the screening tool as it has been demonstrated to be effective (Fucharoen *et al*, 2004; Sanchaisuriya *et al*, 2005; Chaitriphop *et al*, 2016). CMDL-KKU served as a reference center for supporting laboratory diagnosis and in cooperating with obstetricians to undertake PND for the couples at risk.

Data from a university center in Thailand showed that a large number of severe thalassemia diseases can be prevented by implementing a screening system (Yamsri *et al*, 2010). Using the same system, a high

proportion of positive cases was obtained for both pregnant Laotian women and participating spouses. A diverse variety thalassemia genotypes were detected in the pregnant women (Wongprachum *et al*, 2016) as well as among the participating spouses (data not shown). Although there was a low rate (17%) of spouse participation, assessing the risk of carrying a fetus of high risk was 12.5%. The results emphasizes the need for all couples to participate in this type of screening program.

In order to initiate a screening service in a local community, involvement of the local health staff is crucial as they will be part of the program campaign and have to expand the awareness about the disease and its effects to the public (Jopang *et al*, 2015). By offering blood testing for thalassemia and using the results as teaching lessons, the health staff are able to realize that thalassemia is a serious health burden within their community, and this will motivate them even more to be involved in the project. In this study the high rate of staff's participation in blood testing was considered as a key success in getting attention of the other health staff.

For screening procedures, it is essential that the test performance must be acceptable for the target group. Although the overall performance of thalassemia

screening of the local laboratory staff was nearly 100% accurate, an improvement is still needed as a false negative DCIP case was found. In this study, it was difficult to identify the cause(s) of false negative result of this single case as the test was integrated into routine laboratory service. For a better achievement, a dedicated screening unit should be established (WHO, 1994). The screening performance should be monitored regularly through a proficiency testing program. Backup from Thailand was another important factor for planning and referring specimens for diagnosis. With the regular and convenient transportation, all specimens were sent to CMDL-KKU for further investigations. Reporting of the diagnostic results via the internet appeared to work well.

In conclusion, screening for thalassaemia carriers cannot only be performed in middle- but also in low-middle income countries such as Lao PDR, with the cooperation of experienced professionals from (neighboring) middle income countries to set up the scheme. Cooperation and involvement of the local health staff of the institution of the low-income country is most important, in that they will transmit accurate information to the public about thalassaemia and its consequences. However, it must be emphasized that the policy agreed upon and facilities for diagnosis of thalassaemia including PND need to be developed in the country in order to make the program sustainable.

ACKNOWLEDGEMENTS

The work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of Higher Education Commission, through the Health Cluster (SHeP-GMS), Khon Kaen University.

The authors thank all staff of Vientiane Provincial Hospital (Maria Teresa Hospital) and participants for their excellent cooperation.

REFERENCES

- Ainoon O, Cheong SK. Thalassaemia in Malaysia: a strategy for prevention. *Malays J Pathol* 1994; 16: 23-7.
- Chaibunruang A, Prommetta S, Yamsri S, *et al.* Molecular and hematological studies in a large cohort of α (O)-thalassaemia in north-east Thailand: data from a single referral center. *Blood Cells Mol Dis* 2013; 51: 89-93.
- Chaitriphop C, Sanchaisuriya K, Inthavong S, *et al.* Thalassaemia screening using different automated blood cell counters: consideration of appropriate cutoff values. *Clin Lab* 2016; 62: 545-52.
- Chongsuphajaisiddhi T, Salazar N. Role of the SEAMEO TROPED Network in health human resources development. Southeast Asian Ministers of Education Organization Tropical Medicine and Public Health. *Southeast Asian J Trop Med Public Health* 1998; 29: 299-310.
- Choudhuri S, Sen A, Ghosh MK, Misra S, Bhat-tacharyya M. Effectiveness of prenatal screening for hemoglobinopathies in a developing country. *Hemoglobin* 2015; 39: 380-3.
- Fucharoen G, Sanchaisuriya K, Sae-ung N, Dangwibul S, Fucharoen S. A simplified screening strategy for thalassaemia and haemoglobin E in rural communities in south-east Asia. *Bull World Health Organ* 2004; 82: 364-72.
- Fucharoen S, Weatherall DJ. Progress toward the control and management of the thalassaemias. *Hematol Oncol Clin North Am* 2016; 30: 359-71.
- Fucharoen S, Winichagoon P. Thalassaemia in Southeast Asia: problems and strategy for prevention and control. *Southeast Asian J Trop Med Public Health* 1992; 23: 647-55.

- Indaratna K. Screening for thalassemia: an economics viewpoint. *Southeast Asian J Trop Med Public Health* 1997; 28 (suppl 3): 75-81.
- Jaovisidha A, Ajjimarkorn S, Panburana P, Somboonsub O, Herabutya Y, Rungsiprakarn R. Prevention and control of thalassemia in Ramathibodi Hospital, Thailand. *Southeast Asian J Trop Med Public Health* 2000; 31: 561-5.
- Jopang Y, Petchmark S, Jetsrisuparb A, Sanchaisuriya K, Sanchaisuriya P, Schelp FP. Community participation for thalassemia prevention initiated by village health volunteers in northeastern Thailand. *Asia Pac J Public Health* 2015; 27: 2144-56.
- Liao C, Mo QH, Li J, *et al.* Carrier screening for alpha- and beta-thalassemia in pregnancy: the results of an 11-year prospective program in Guangzhou Maternal and Neonatal hospital. *Prenat Diagn* 2005; 25: 163-71.
- Sanchaisuriya K, Fucharoen S, Fucharoen G, *et al.* 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.
- Savongsy O, Fucharoen S, Fucharoen G, Sanchaisuriya K, Sae-ung N. Thalassemia and hemoglobinopathies in pregnant Lao women: carrier screening, prevalence and molecular basis. *Ann Hematol* 2008; 87: 647-54.
- Tekola-Ayele F, Rotimi CN. Translational genomics in low- and middle-income countries: opportunities and challenges. *Public Health Genomics* 2015; 18: 242-7.
- Tritipsombut J, Sanchaisuriya K, Phollarp P, *et al.* Micromapping of thalassemia and hemoglobinopathies in different regions of northeast Thailand and Vientiane, Lao People's Democratic Republic. *Hemoglobin* 2012; 36: 47-56.
- Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. *Blood* 2010; 115: 4331-36.
- William TN, Weatherall DJ. World distribution, population genetics, and health burden of the hemoglobinopathies. *Cold Spring Harb Perspect Med* 2012; 2: a011692.
- Winichagoon P, Thitivichianlert A, Lebnak T, Piankijagum A, Fucharoen S. Screening for the carriers of thalassemias and abnormal hemoglobins at the community level. *Southeast Asian J Trop Med Public Health* 2002; 33 (suppl 2): 145-50.
- Wongprachum K, Sanchaisuriya K, Dethvongphanh M, *et al.* Molecular heterogeneity of thalassemia among pregnant Laotian women. *Acta Haematol* 2016; 135: 65-9.
- World Health Organization (WHO). Guidelines for the control of haemoglobin disorders. *WHO/HDP/HB/GL/94.1*, 1994.
- World Health Organization(WHO). WHO country cooperation strategy for the Lao People's Democratic Republic 2012-2015. Geneva: WHO, 2011.
- Yamsri S, Sanchaisuriya K, Fucharoen G, Sae-Ung N, Fucharoen S. Genotype and phenotype characterizations in a large cohort of β -thalassemia heterozygote with different forms of α -thalassemia in northeast Thailand. *Blood Cells Mol Dis* 2011; 47: 120-4.
- Yamsri S, Sanchaisuriya K, Fucharoen G, Sae-Ung N, Ratanasiri T, Fucharoen S. Prevention of severe thalassemia in northeast Thailand: 16 years of experience at a single university center. *Prenat Diagn* 2010; 30: 540-6.