PILOT SCREENING PROGRAM FOR THALASSEMIA CARRIERS AT COMMUNITY LEVEL IN LAO PEOPLE'S DEMOCRATIC REPUBLIC

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Abstract. A pilot screening program for thalassemia carriers at a community level was launched in six villages of Keo-Oudom District, Vientiane Province, Lao People's Democratic Republic (Lao PDR). Prior to implementing the program, essential facilities including manpower, laboratory facilities and referral system for detailed analysis of blood samples were developed. Knowledge and attitude towards thalassemia among 171 Laotian women of reproductive age were evaluated before and after providing the education program. There is a significant increase in knowledge and positive attitude regarding thalassemia following completion of the program in both scores. All participants accepted to have an on-site blood test, which revealed a carrier frequency of 15%, 5% and 24% for α^0-thalassemia, β-thalassemia and hemoglobin E, respectively. Subsequent analysis at the referral center identified the presence of Hb H, Hb H with Hb Constant Spring (CS), EABart's, EABart's with Hb CS, and an unusual form of Hb E-β-thalassemia. These findings indicate the feasibility of implementing a screening program for thalassemia carriers at the community level in Lao PDR and emphasize the complexity of thalassemia genotypes among Laotian people.

Keywords: thalassemia, screening program, education program, Vientiane, Lao PDR

INTRODUCTION

Thalassemia, a group of autosomal recessively inherited hemoglobin disorders, remains poorly recognized by health authorities in many developing countries where prevalence of carriers of this syndrome is polymorphic. In Southeast Asia, not only α-thalassemia (α-thal) and β-thalassemia (β-thal) but also hemoglobin E (Hb E), a thalassemic hemoglobin variant, are common (Fucharoen and Winichagoon, 1992; Weatherall and Clegg, 2001). The thalassemias manifest a variety of clinical severity, which not only need medical attention but also constitute a public health burden to the economic and impose social strains for the affected communities. In Thailand these issues have been recognized for quite some time and attempts are underway to prevent

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the occurrence of thalassemia through integration of this problem into primary health care structure (Jopang *et al*, 2015).

In the Lao People's Democratic Republic (Lao PDR), previous investigations have shown similar features of thalassemia to those seen in northeast Thailand (Savongsy *et al*, 2008; Tritipsombut *et al*, 2012; Wongprachum *et al*, 2016a). A higher prevalence and complexity of thalassemias in Lao PDR, indicates the occurrence of children born with the three severe forms of the disease, namely Hb Bart's hydrops fetalis, β-thalassemia major and compound heterozygosity of β-thalassemia and Hb E (Hb E-β-thal), must be higher in comparison to Thailand (Tritipsombut *et al*, 2012). With the assistance of Thai universities, health authorities of Lao PDR have become aware of this threat of thalassemia to the nation's public health and have initiated a pilot screening program for thalassemia with the goal of collecting data for planning of preventive measures (Wongprachum *et al*, 2016a; *ibid*, 2016b).

Due to a lack of facilities for laboratory and prenatal diagnosis, implementing antenatal screening may not be achievable in Lao PDR. Premarital screening or screening in women of reproductive-age, and providing them information for family planning might be alternative options. Whether or not such an approach is feasible requires assessment of perception toward thalassemia in the target group. In this study, we assessed the perception toward thalassemia among Laotian reproductive-age women living in six villages of rural Vientiane. Blood testing for thalassemia was provided to participants and considered as the main outcome determining the feasibility of implementing screening program for thalassemia at the community level. Results of the blood test were also reported to the participants.

MATERIALS AND METHODS

Participants

The survey was conducted in six villages of the Keo-Oudom District within the rural area of Vientiane Province, Lao PDR. Accessibility to the villages was one major selection criterion. Educational materials, *eg*, pamphlets and flipcharts, were translated into the Lao language from the Thai versions (Jopang *et al*, 2015). Thalassemia education program was provided to local health care workers (HCWs) including village health volunteers (VHVs). A trained doctor from Vientiane Provincial Hospital was the lecturer. The ability of VHVs to convey correct information to the community was assessed through a pre- and post-test.

Prior to conducting the study, ethical approval was obtained from the National Ethics Committee for Health Research of Lao PDR (NIOPH/NECHR No.055) and the Ethics Committee of the Khon Kaen University, Thailand (HE582179).

Education program protocol

The project was announced to the target communities via the village broadcasting system. All reproductive-age women volunteering to participate in the project were invited to a meeting at the village meeting hall. The participants were informed about the details of the program, and upon obtaining informed consent, demographic information of the participants was collected. Prior to providing the education program, knowledge and perception about thalassemia among participants were assessed by using questionnaires, which had been used in the northeast Thailand (Jopang *et al*, 2015).

The questionnaires were adapted to the context of Lao PDR. Knowledge and attitudes toward thalassemia were assessed again at the end of the education session.

Basic knowledge provided to participants included 1) thalassemia disease, 2) need for a blood test to identify a thalassemia carrier, 3) healthy status of a thalassemia carrier, 4) that a baby may be affected if both parents are carriers, 5) that if both parents are carriers, the chance of having an affected baby is one in four for each pregnancy, 6) the best way to prevent a baby from severe thalassemia disease is that married couples should have blood tests before pregnancy, 7) in the case of pregnancy where both parents are thalassemia carriers, prevention of having an affected baby includes antenatal screening, prenatal diagnosis and termination of pregnancy, and, 8) a thalassemia patient can be treated by regular blood transfusion (and iron chelation), splenectomy and bone marrow transplantation. These eight items of knowledge were posed in the form of questions. A score of one was given for each correct answer.

For attitude assessment, questions were asked regarding whether or not participants agreed or disagreed with the topic. Main attitude inquiries included perception of participation in blood testing, willingness to perform prenatal diagnosis (PND) and to undergo termination of pregnancy. Eight-item attitude questionnaire comprised both positive and negative questions. For positive questions, a score of "1" was given for 'agree' and a score of "0" for 'disagree' answer. Conversely, for negative questions, a score of "1" was given for 'disagree' answer and "0" for 'agree' answer.

At the conclusion of the project, diagnostic results and in-depth one-on-one counseling regarding thalassemia status were provided to all participants. On the day of information of the diagnostic results, perception towards thalassemia was assessed again, using the same questionnaire.

Thalassemia screening and diagnosis

Screening for thalassemia was offered to participants after providing the education program. Blood samples collected from participants initially were screened for thalassemia at the Vientiane Provincial Hospital. Red blood cell indices were measured using an automated hematology analyzer (HumaCount, Wiesbaden, Germany) to identify individuals suspected as carriers of α^0-thal or β-thal. Hb E carrier was identified using the KKU-DCIP-Clear reagent kit (PCL Holdings, Bangkok, Thailand). Positive screening was considered according to the combined tests of mean corpuscular volume and DCIP test (MCV/DCIP). Cutoff value for positive MCV is set at <78 fl (Chaitriphop *et al*, 2016). Following performing the screening tests, all residual blood samples were referred to the Center for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Science, Khon Kaen University, Thailand, for confirmation. Standard methods including hemoglobin and DNA analyses were performed. Cellulose acetate electrophoresis (Helena Laboratories, Beaumont, TX) was used to determine hemoglobin type. Quantification of Hb A_2 was performed in all cases with MCV <78 fl, using an automated capillary zone electrophoresis (Capillarys II; Sebia, Lisses, France). β-thal carrier was diagnosed when Hb A_2 >4.0% (Yamsri *et al*, 2011). Identification of α^0-thal mutation was performed in all samples using gap-PCR for SEA and THAI deletion genotypes (Sae-ung *et al*, 2007). Positively-screened individual who has

Table 1
General characteristics of participants.

Characteristic	Number Total=171 (%)
Age (years)	
<20	23(13)
20-35	92(54)
>35	56(33)
Marital status	
Single	41(25)
Married	127(74)
Divorced	3(2)
Education level	
Primary school or lower	52(31)
Secondary school	103(61)
Vocational school	9(5)
Bachelor degree or higher	7(4)
Occupation	
Farmer	86(50)
House wife	20(12)
Government employee	16(9)
Student	24(14)
Others (Laborer and trader)	25(15)
Family income (Kip/month)	
< 1,000,000	46(27)
1,000,000-3,000,000	113(66)
> 3,000,000	12(7)
Family history of thalassemia	
Yes	8(5)
No	163(95)
Heard about thalassemia before	
Yes	107(63)
No	64(37)
Source of thalassemia information	
Mass media (TV and radio)	41(38*)
Person (health professionals/friends)	26(24*)
Both media and person	41(38*)

*Total *n*=108.

Hb A2 < 4.0% and negative DNA analysis for α^0-thal mutation is concluded as having non-clinically significant thalassemia, whereas an individual with negative screening as either non-thalassemia or non-clinically significant thalassemia (Fucharoen *et al*, 2004).

Data analysis

Data were analyzed using the STATA/IC 10.0 for Windows (STATA Corp, College Station, TX). Variables presenting general characteristics of participants were described using mean ± SD or percentage where appropriate. Differences in mean scores of knowledge and attitude towards thalassemia before and after education were compared statistically using paired *t*-test. Chi-square test was used to test the association between proportion of correct answer and demographic characteristics. Hematological parameters among participants with different forms of thalassemia were compared to non-thalassemia group using independent *t*-test. Statistically significant difference is set at a *p*-value <0.05.

RESULTS

A total of 171 reproductive-age women volunteered to participate. The majority of participants were in the age group of 20-35 years or higher, were married and were secondary or primary school graduates, and had a family income of 1,000,000-3,000,000 Kip (USD 123-370)/month (Table 1). Half of the participants were famers. Eight women (5%) reported a family history of thalassemia and 63% of the participants had heard about thalassemia.

There is a significant increment in scores for both knowledge and attitude following the education program (Table 2). As regards knowledge of thalassemia, the majority of participants answered correctly that 'blood test is needed to identify carrier'; and for attitude towards thalassemia, majority agreed upon all positive questions, including agreement to have blood test even if healthy and to consult physician if wishing to having

Table 2
Assessment of knowledge and attitude regarding thalassemia before and after education among 171 Laotian women of reproductive age.

Category*	Before	After
Knowledge	4.0 ± 1.0	5.8 ± 0.8**
Attitude	5.9 ± 1.4	7.3 ± 0.9**

*Total score = 8. **p-value <0.001 (paired t-test).

a thalassemia child (Table 3). Many participants also agreed to bring their partner to consult a physician during pregnancy if both are carriers and have PND if the physician suggests so. More than half of the participants disagreed with termination of pregnancy when asked prior to the education program, but 97% of thalassemia carriers agreed with this issue after knowing their thalassemia status (data not shown). The proportion of participants who answered each question correctly or had positive attitudes is increased significantly in the post-test (Table 4).

Every participant agreed to have a blood test and 81 (47%) were positive in the preliminary thalassemia screening, among whom 67 (83%) subsequently were confirmed to be thalassemia carriers, with 15%, 4% and 23% of the participants being α^0-thal, β-thal and Hb E carriers, respectively (Table 5). A small number of women with non-transfusion dependent thalassemia (NTDT), including Hb H, Hb H with Hb Constant Spring (CS), EABart's, EABart's with Hb CS and Hb E-β^0-thal were also identified. Among NTDT individuals, Hb levels varied from 5.3 g/dl in a case of Hb E-β-thal to 9.2 g/dl in Hb H disease.

DISCUSSION

Other studies have shown that knowledge and attitude scores are associated with education, income and occupation (Wong *et al*, 2011a; *ibid*, 2011b). In this study, we found no association because the levels of socio-economic status and educational background of participants were quite uniform. In general, the majority of participants knew that thalassemia is an inherited disease and can be diagnosed by blood test. However, they were unaware that thalassemia carrier can be healthy. Similar to this study, other investigators reported the same misconception (Wong *et al*, 2011a). Even after education, this misconception continued to exist among almost half of the participants. This is probably one of the major stumbling block for the general population to fully recognize the hidden danger and possibility of severity of the disease. Strong community organization and leadership should be encouraged to take part in health education for preventing and controlling thalassemia.

Whether or not carrier individuals accept termination of pregnancy was also a key issue to be considered. That nearly all participants who became aware that they are carriers of thalassemia were positive towards this issue indicates that an individual's attitude towards abortion was more likely to change when one is directly affected. This underlines the importance of population screening program and emphasizes the role of HCWs in conveying the message to the community as well as providing appropriate counseling to the affected individuals.

False-positive rate of the preliminary thalassemia screening was low (<20%). Similar to Thai population (Winicha-

Table 3
Correct answers regarding thalassemia by 171 Laotian women of reproductive age before and after participating in education program.

Knowledge item	Before, *n* (%)	After, *n* (%)
1. Thalassemia is a hereditary disease.	112 (65)	164 (96)
2. Knowing a carrier status requires blood test.	163 (95)	168 (98)
3. A thalassemia carrier can be healthy.	22 (13)	83 (48)
4. A baby may be affected if both parents are carriers.	90 (53)	133 (78)
5. If both parents are carriers, a chance of having an affected baby is one in fourth.	66 (39)	105 (61)
6. The best way to prevent a baby from severe thalassemia disease is 'the couples should have a blood test before pregnancy'.	120 (70)	147(86)
7. In case of pregnancy, prevention approach includes antenatal screening, prenatal diagnosis and termination of pregnancy.	34 (20)	80 (47)
8. A thalassemia patient can be treated by regular blood transfusion (and iron chelation), splenectomy and bone marrow transplantation.	81 (47)	111 (65)

Table 4
Positive attitudes towards thalassemia of 171 Laotian women of reproductive age before and after participating in education program.

Attitude item	Before, *n* (%)	After, *n* (%)
1. Willingness to have a blood test, if healthy.	160 (93)	169 (99)
2. Willingness to have a blood test, if no relatives with thalassemia disease.	97 (57)	143 (84)
3. Willingness to take a partner to screen for thalassemia, if carrying thalassemia gene.	69 (40)	139 (81)
4. Willingness to take a partner to consult the physician during pregnancy, if both are thalassemia carriers.	146 (85)	165 (96)
5. Willingness to have PND, if the physician suggests so.	150 (88)	164 (96)
6. Willingness to terminate the pregnancy, if carrying a child with severe thalassemia.	80 (47)	138 (81)
7. Willingness to consult the physician for family planning, if having a thalassemia child.	156 (91)	165 (96)
8. Willingness to have no children, if carrying thalassemia gene.	92 (54)	149 (87)

goon *et al*, 2002; Fucharoen *et al*, 2004; Sanchaisuriya *et al*, 2005; Sangkitporn *et al*, 2005; Tritipsombut *et al*, 2012), the estimate prevalence of severe thalassemia syndromes in the Laotian population is 6/1,000 for homozygous α^0-thal or Hb E-β-thal and 5/10,000 for homozygous β-thal. Although treatment may not be necessary for α^0-thal homozygote (Hb Bart's hydrops fetalis) as the disease leads to fetal death, an affected mother may require therapeutic abortion to

Table 5
Hematological picture and thalassemia status of 171 Laotian women of reproductive age.

MCV/DCIP (*n*)	Diagnosis	*n*	RBC (10^{12}/)	Hb (g/dl)	MCV (fl)	MCH (pg)	RDW (%)	Hb type	Hb A_2/E (%)	Hb F (%)
+/- (36)	α^0-thal trait	14	5.67 ± 0.33*	11.5 ± 0.6*	60.2 ± 3.4*	20.2 ± 1.1*	15.5 ± 0.8*	A_2A	2.2 ± 0.3	0.1 ± 0.2
	β-thal trait	7	5.7 4 ± 0.38*	11.0 ± 0.7*	56.1 ± 2.8*	19.2 ± 1.0*	16.3 ± 0.9*	A_2A	5.6 ± 0.6	1.9 ± 2.3
	Hb H disease	2	5.08, 6.05	8.7, 8.9	42.0, 53.0	14.6, 17.2	28.1, 19.1	A_2ABart'sH	1.2, 1.8	0, 0.3
	Hb CS carrier	1	4.71	10.3	66.8	22.0	14.0	CSA_2A	1.7	0.7
	Suspected non-clinically significant thal	12	5.21 ± 0.58*	12.5 ± 1.6	71.2 ± 7.80*	21.5 ± 3.0*	15.2 ± 1.9*	A2A	2.3±0.4	0
+/+ (29)	Hb E trait	15	5.12 ± 0.57*	12.3 ± 1.3*	70.3 ± 2.8*	24.1 ± 0.6*	14.6 ± 0.7*	EA	n.d	n.d
	Hb E trait with α^0-thal	6	5.43 ± 0.22*	11.2 ± 0.3*	60.7 ± 3.6*	20.7 ± 0.9*	15.6 ± 1.0*	EA	n.d	n.d
	Homozygous Hb E	2	5.83, 5.95	11.6, 11.9	58.2, 61.0	19.6, 20.4	15.8, 17.1	EE	n.d	n.d
	Hb H-CS disease	2	3.13, 5.38	7.0, 9.2	58.0, 74.0	17.1, 22.4	17.8, 22.1	CSA_2ABart'sH	0.8, 0.5	0
	Hb E trait with Hb H disease[a]	1	6.72	9.4	43.1	14.1	21.6	EA	15.5	0
	Hb E trait with Hb H-CS disease[b]	1	5.02	7.4	51.3	14.7	23.7	CSEABart's	14.8	1.8
	Hb E-β-thal disease	1	3.62	5.3	45.4	14.7	26.3	EE	93.1**	6.9
	Hb CS carrier	1	5.27	12.3	71.1	23.4	14.2	CSA_2A	2.2	0
-/+ (16)	Hb E-trait	14	4.85 ± 0.36*	12.6 ± 0.8	76.2 ± 3.2*	26.0 ± 1.0*	13.8 ± 0.3	EA	n.d	n.d
	Suspected non-clinically significant thal	2	5.19, 4.5	13.6, 12.0	76.9, 75.9	26.1, 26.7	13.1, 14.1	A_2A	n.d	n.d
-/- (90)	Non-thal or non-clinically significant thal	90	4.59 ± 0.40	12.7 ± 0.9	80.7 ± 4.1	27.8 ± 1.4	13.7 ± 0.6	A_2A	n.d	n.d

n.d, not determined. [a]So-called EABart's disease. [b]So-called EABart's-Hb CS disease. *p-value <0.01 compared to normal. **Hb E = 83.8%, Hb HbA_2 = 9.3%. MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; RDW, red blood cell distribution width.

prevent serious complications including preeclampsia, postpartum hemorrhage, and premature delivery (Liang *et al*, 1985; Suwanrath-Kengpol *et al*, 2005; Yang and Li, 2009; Vichinsky, 2013). The existence of Hb E-β^0-thal and homozygous β^0-thal individuals results in socio-economic burden not only for the family concerned but also the nation as these patients require life-long treatment and care.

A participant with atypical characteristics of Hb E-β-thal was identified. The individual has severe anemia but never received blood transfusion. Most importantly, the hemoglobin profile is similar to that of homozygous Hb E, and this may lead not only to misdiagnosis of the disease but also to inappropriate counseling for the family. A similar finding was reported in a pregnant Laotian woman (Wongprachum *et al*, 2016a), indicating that such cases may not to be uncommon among the Lao people. Several factors, which modify the clinical severity of thalassemia, are now known (Sherva *et al*, 2010; Winichagoon *et al*, 2000; Rujito *et al*, 2016). This information is crucial for HCWs to recognize the complex nature of thalassemia within their communities. Our findings emphasize again that thalassemia is a serious public health problem for Laotian population and certainly justify planning and implementation of an appropriate prevention strategy.

A prevention approach could be adopted as in Thailand where all pregnant women and their partners are encouraged to be screened while attending antenatal care service (Fucharoen and Winichagoon, 1992). Either MCV or osmotic fragility test is used in combination with the DCIP test to identify carriers of three clinically significant thalassemia carriers, namely, α^0-thal, β-thal and Hb E. Blood samples of positive couples are then referred to a clinical center for further investigation. Genetic counseling and prenatal diagnosis are provided to at-risk couples. By offering the choice of termination of pregnancy, birth of a large number of affected fetuses are prevented (Yamsri *et al*, 2010). Due to a lack of facilities for laboratory and prenatal diagnosis, implementing antenatal screening as conducted in Thailand may not yet be fully achievable in Lao PDR.

In summary, we initiated for the first time a pilot screening program for thalassemia carriers among non-pregnant women of reproductive age in 6 villages of rural Vientiane. Even though there was lack of laboratory facilities for molecular diagnosis, it was possible to operate a thalassemia screening program in Lao PDR with the cooperation of a referral center located within close proximity in Thailand (Wongprachum *et al*, 2016b). A better understanding and positive attitude toward thalassemia achieved after providing an education program together with a good cooperation in blood testing confirmed the feasibility of implementing a prevention program for thalassemia at the community level in Lao PDR.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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