Preliminary Study on Thalassemia Screening and Genetic Counseling in Selective Hmong People in Saraburi Province, Thailand

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ABSTRACT

Thalassemia is a gene-linked disease that can cause serious health problems because it can lead to the destruction of red blood cells. Studies have shown that there is a high prevalence of thalassemia in Southeast Asia. The Institute of Health Research, Chulalongkorn University developed a successful "Module" to screen for thalassemia in the Thai population, however, it has not been implemented in the minority population in Thailand. In this study, we investigated the feasibility of the newly developed educational and thalassemia screening program with the Hmong population. The primary aim of this study was to test this program. The secondary aim was to determine the prevalence of thalassemia in the Hmong and provide education. A third aim was to determine the reliability of two different screening methods in the Hmong population. A pre-test and post-test design was used; participants (N=12) were individuals residing in Thailand with the ability to read English and between the ages 18-50. The participants met twice with the researchers to complete the program. The first contact consisted of assessing participants' knowledge about thalassemia, providing thalassemia information and education about genetic counseling, and drawing blood samples. The second contact consisted of assessing knowledge, providing a written report of individual blood sample results and counseling. The initial interview revealed that the majority of the participants (82%) did not know anything about thalassemia prior to participation. The program was easy to understand by most participants (90%). Of the eleven Hmong participants, two tested positive for being a possible carrier for thalassemia. In order to reduce the prevalence of thalassemia, it is necessary to engage in risk reduction health services. The modified screening method proved to be as effective as the standard method. Therefore, the program can expand and be used in other regional populations with low cost.

INTRODUCTION

Thalassemia is the most common single gene disorder worldwide. It is an inherited blood disorder that reduces the amount of hemoglobin the body makes and leads to the destruction of red blood cells. A person can only get thalassemia trait or disease by inheriting the genes for thalassemia from their parents. The common signs and symptoms of thalassemic diseases include pale skin, retarded growth and puberty, anemia, enlarged spleen, and increased susceptibility to infections.

In this study, we investigated the feasibility of the newly developed educational and thalassemia screening program with the Hmong population. The primary aim of this study was to test this program. The secondary aim was to determine the prevalence of thalassemia in the Hmong and provide education. A third aim was to determine the reliability of two different screening methods in the Hmong population.

In Thailand, thalassemia occurs with high incidence and presents individual, social and economic burdens (Chareonkul & Kraisin, 2004; Winichagoon et al., 2002). It is estimated that 1% of Thai people have the disease and that 30-40% of Thai people carry abnormal genes that can be transmitted to their offspring (Pansatiankul & Saisorn, 2003). Of the married couples in Thailand, approximately 18-20% are at risk for giving birth to infants with inherited mutations (Winichagoon et al., 2002). The high prevalence of thalassemia makes it a serious public health concern. Therefore, it is important to study prevention and effective education and screening materials to decrease the prevalence of thalassemia.

Thalassemia can be controlled through engaging in preventive health services. Health education is incorporated to raise awareness and to motivate clients to seek available services. Education about the risks of conceiving a child with thalassemia combined with existing family planning services is an effective approach to inform the behaviors of those at risk for thalassemia. Laboratory blood tests can determine the status of thalassemia in a person. Screening and genetic counseling have been associated with numerous benefits, including a decreased number of thalassemic newborns (Chareonkul & Kraisin, 2004). The aim of screening for thalassemia 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 (Fucharoen et al., 2004).

The Institute of Health Research (IHR), Chulalongkorn University has developed a program for the screening of thalassemia traits (Sindhuphak et al., 2000) and genetic counseling for voluntary contraception (Dusitsin et al., 1998). The education and screening program is inexpensive and simple to learn. The program consists of the following: (1) thalassemia education to raise public awareness, (2) screening by conducting appropriate tests relevant to thalassemia (3) genetic counseling using the black and white rabbit mendelian chart and (4) referrals when necessary.

The program has been highly reliable in identifying most individuals who are carriers among the Thai population. However, the program has not been used in the Hmong population. The Hmong are an isolated minority group from southern China that has, over the years, migrated to the mountainous regions of Vietnam, Cambodia,

Laos and Thailand. There has been little research about this population. The thalassemia prevalence rate in the Hmong population is unknown. Therefore, the screening for thalassemia status in the Hmong population is indicated not only for improving the health status of this population, but also for early intervention and prevention of this disease. If we can disseminate thalassemia knowledge and determine the prevalence of thalassemia in Hmong people, then we can develop effective screening and genetic counseling methods to help them have knowledge about thalassemia. An increased knowledge in decision making about childbearing can prevent and/or control new cases. In turn, we would reduce the transmission of this disease.

The standard screening blood tests OF and DCIP involved sending the samples to a central location, which was costly. The modified screening blood tests were developed to provide inexpensive and quick screening results in the field. Thus, the third aim was to test the reliability of the modified screening blood tests to the standard.

METHODS

Procedure

Our study was approved by the Chulalongkorn University ethics committee. Participants were recruited through electronic mail advertisements and by personal contacts. A participation information sheet was given to each participant and any questions they had were answered. Each participant signed informed consent forms. The participants completed a pre-questionnaire. Thalassemia information and a genetic counseling kit were presented to each participant. Afterwards, the

participants completed a post questionnaire. At the end of the first contact, 3mL of peripheral blood anticoagulated with ethylenediaminetetraacetic acid (EDTA) was obtained from each participant. The blood samples underwent blood screening tests (OF and DCIP) at the Institute of Health Research, Chulalongkorn University. The blood samples were also delivered to Chulalongkorn Hospital for Hb typing and Red Blood Cell Indices analyses. A negative OF and negative DCIP result indicated that the participant was not a carrier of thalassemia. A positive result from either OF and/or DCIP indicated that the participant was a possible carrier for thalassemia. Once all of the screening blood tests were completed by IHR and Chulalongkorn Hospital, participants received a written report of individual results during the second contact.

Design

The study was carried out in Saraburi Province, Thailand. This province is about an hour north of Bangkok, Thailand. A pre-test and post-test design was used. The participants completed two questionnaires on two different occasions. Questionnaire one included demographic data, family history about thalassemia, and their knowledge of thalassemia prior to participating in the project. Questionnaire two included knowledge of thalassemia after the intervention and their understanding of the hereditary transmission. Eligibility criteria included Hmong individuals residing in Thailand with the ability to read English and between the ages 18 to 50. We recruited English speaking people because the program was translated to English. We focused on this age range because of the reproductive stage.

Education and genetic counseling protocol

The protocol translated to English language included IHR's Thalassemia Information flip chart with a diagram of probability of inheritance of thalassemia in the offspring, probability cards and pamphlets. The information was comprised of thalassemia epidemiology, symptoms, characteristics and treatment options. This protocol is user-friendly for explaining the mechanism of thalassemia hereditary transmission from parents to their offspring. For example, the information about dominant and recessive traits was presented in picture form. A white rabbit represented a dominant trait. A black rabbit represented a recessive trait. The black and white rabbit cards were used to understand the probability of having a normal or afflicted offspring in each pregnancy. Participants drew a card from a pack of four shuffled relevant probability cards to see their offspring chances.

Screening tests

One Tube Osmotic Fragility Test (OF): The "Thal screen solution" was obtained from Chiang Mai University. One drop of mixed whole blood was added to 10ml of "Thal screen solution" and mixed by inserting a stopper on top of the tube and inverting 4-5 times. The mixture was left at room temperature for 10 minutes to allow hemolysis. The mixture solution can be kept up to 3 hours before reading. A negative result is indicated if the mixture solution is clear and when the tube is placed before a typescript, the typed letters can be clearly visualized through the solution. A result that is turbid where the typescript cannot be visualized through the solution is read as positive.

Dichlorophenol Indophenol Precipitation (DCIP) test: KKU-DCIP-Clear reagent kit was obtained from KhonKaen University. One drop of mixed whole

blood was added to 2ml of KKU-DCIP-Clear Reagent and mixed by inserting a stopper on top of the tube and inverting 4-5 times. The mixture was incubated at 30°C for 15 minutes without agitation. The tube was then removed from incubation and one drop of clearing solution (clearing powder in 1ml of sterile distilled water) was added to the solution and mixed. The result could be readily seen after allowing the mixture to stand 2-3 minutes via the light. A negative result is indicated by a clear solution. A result that is turbid is read as positive.

Red Blood Cell indices: Determination of erythrocyte indices using the Technicon H*3 automated blood cell Counter at Cholalongkorn Hospital's laboratory.

Confirmatory test

Hb typing: Types of Hb were determined using standard cellulose acetate electrophoresis at Chulalongkorn hospital's laboratory.

Validation of screening method

The researcher was trained to perform the modified screening methods OF and DCIP. The results of all tests were evaluated by standard screening methods at IHR's laboratory and the screening efficiency was calculated accordingly.

RESULTS

Sample

The sample consisted of twelve subjects, of whom 11 were Hmong and one was Laotian. The Laotian participant was a spouse of a Hmong participant, therefore, his/her results were relevant to the screening and genetic counseling portion of the program. The Laotian participants' results were not used in determining the

prevalence rate of thalassemia in Hmong. Two of the participants were men and ten were women. Two participants were born in the United States of America, three of the participants were born in Thailand, and seven were born in Laos. All of the participants were residing in Saraburi Province, Thailand. The average age of participants was 31 years. For eleven participants, a college or university degree was the highest level of education obtained, and for one a master's degree as shown in Table 1.

Everyone invited to participate in the research accepted. Eleven participants completed the pre-questionnaire. Eleven participants actively participated in the genetic counseling portion of the study. Twelve blood samples were obtained from participants including the researcher due to her being Hmong. Ten participants completed the post-questionnaire. One participant was not available to complete the post-questionnaire.

Family knowledge

Six participants did not know anyone in their family who had thalassemia. Five participants answered that none of their family members had thalassemia. Nine of the participants planned to have children; two participants responded they did not plan to have children. Eight participants were single and four were married. Of the four married participants, two participants responded they do not use any form of contraception, one responded to the use of condoms and one stated the use of abstinence.

Thalassemia knowledge

Only two participants knew about thalassemia prior to participation in this study. After the education intervention, the participants acquired basic knowledge of thalassemia. As shown in Table 2, participants' knowledge scores increased after the intervention. Figure 1 shows that more than half of the participants correctly answered the genetic counseling questions of the questionnaire.

Genetic counseling education

To evaluate the effectiveness of the counseling education, participants were questioned on their understanding of thalassemia transmission. As displayed in Table 3, 70% of the participants reported that the kit was easy to understand and 30% reported that they understood the kit fairly well. Further, 20% reported that they could explain the kit to others and 80% reported that they could somewhat explain the kit to others. More than 80% of the participants correctly answered the probability of offspring inheritance for thalassemia. All of the participants reported that they would like to have the rabbit charts for genetic counseling available.

Post education control and prevention opinions

80% of the participants reported that the best way to control and prevent thalassemia would be to have a blood test before marriage and a blood test before pregnancy. 60% reported using contraception and family planning and one participant report having a blood test after pregnancy. 80% of the participants reported that they would use contraception and family planning.

Blood tests

The results of screening for Thalassemia and Hb E using modified OF and DCIP, the haematological analysis of 12 participants are shown in Table 4.

Negative results were found in nine Hmong participants. Screen tests, OF and DCIP showed relatively normal haematological parameters including MCV (>80 fl), MCH (>27 pg). For these only Hb typing was analysed and no β-thalassemia or Hb E carriers were identified.

Only two participants with a positive result of OF and negative result of DCIP had Hb A_2A. The erythrocyte indices including MCV and MCH values of one participant were lower than nine participants with negative screening tests.

The spouse of a Hmong participant, who is Laotian, had positive results on both OF and DCIP and relatively lower haematological parameters including MCV and MCH than normal. Hb analyses showed the result is Hb E carrier, indicating high sensitivity and specificity for the DCIP test used for Hb E detection.

Modified screening methods

The results of using a modified screening procedure and standard screening methods were not different as shown in Table 5. The effectiveness of the screening strategy is shown in Table 6. Based on only Hb typing, we found that both OF and DCIP had high sensitivity and specificity.

DISCUSSION AND CONCLUSION

There is a high prevalence of thalassemia in some tropical parts of the world and in some of the richer industrialized countries because of immigration. The control and prevention programs for thalassemia have been established and successful in many countries consisting of education, counseling, screening and antenatal diagnosis. Thailand and many developing countries have undergone a demographic transition; provision of service is still hampered by major economic and

organizational difficulties. Therefore, development of screening strategies and genetic counseling materials are necessary.

This study reveals that a newly designed education and screening program is feasible among the Hmong population in Thailand. Everyone accepted the invitation to participate. The pre-questionnaire showed that Hmong are presently unaware of thalassemia or have little knowledge about this disease, but they are willing to learn and can easily understand the information provided. Participants' knowledge of thalassemia and screening methods improved, as did the understanding of genetic transmission.

Modified OF and DCIP by IHR proved to be easily performed at selected primary health centers in Thailand. Both blood testing and the counseling guidelines proved to be simple and low cost. All participants completed a blood test. Participants were eager to learn about thalassemia and satisfied with the services provided.

Two of the eleven Hmong participants (18%) tested positive for being a possible carrier for thalassemia. Therefore, it is necessary to provide the Hmong population with relevant information about thalassemia in order to increase their awareness and knowledge of the condition. Early intervention can provide prevention of this disease. Screening and genetic counseling programs are important because both parents may be carriers, creating a significant risk for a future baby to have a major hemoglobin disorder.

The program was successful and well accepted in the Hmong sample. It is suggested that screening and counseling should be expanded into other regional

centers for further testing. The modified blood screening was equal to the standard blood screening. This supports the validity of a modified test. This is important because the screening is easier to use and less expensive, therefore, facilitating screening programs in local health care areas.

We recognize some limitations in our study. There was little relevant literature that could be found about the Hmong population. In addition, no literature was available regarding thalassemia in the Hmong population. The study was conducted in English. Our sample was limited in size (N=12), diversity of race and geographic representation. Thus, our findings are most applicable to Hmong people living in Thailand with the ability to read English.

Future research can examine a larger Hmong population to detect and determine the prevalence of thalassemia. Researchers can learn about the prevalence status in order to improve health status. This new program could be replicated in other minority group samples in Thailand. It could also be replicated with English speaking Hmong groups in the United States.

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Table 1. Demographic (N=12)

Demographic	#	%
Gender		
Male	2	17
Female	10	83
Age		
22-30	6	50
31-42	6	50
Country of birth		
Thailand	3	25
Laos	7	58
United States of America	2	17

Demographic	#	%
Marital status		
Single	8	67
Married	4	33
Education		
College/University	11	92
Graduate School	1	8
Religion		
Buddhism	1	8
Christian	5	42
Catholic	1	8
Shamanism	5	42

Table 2. Comparison of Pre- and Post- knowledge about Thalassemia

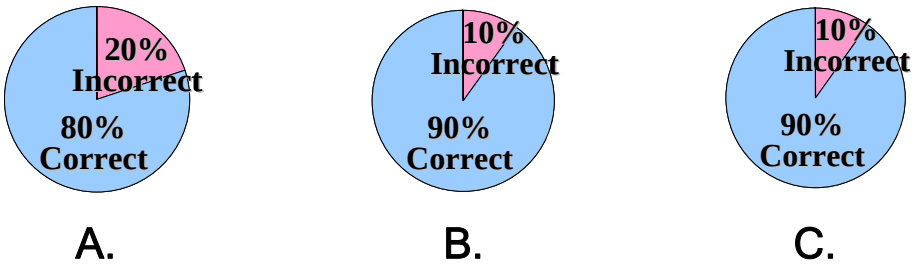
Questions	**Pre-questionnaire** (n=11)		**Post-questionnaire** (n=10)	
	#	%	#	%
Thalassemia is a disease associated with abnormal red blood cells.				
Yes	3	27	8	80
No	0	0	1	10
Don't know	8	73	1	10
Thalassemia is an inherited disease.				
Yes	6	54	10	100
No	0	0	0	0
Don't know	5	46	0	0
Blood tests can tell me if I am a thalassemia carrier.				
Yes	7	64	10	100
No	0	0	0	0
Don't know	4	36	0	0
The symptoms of thalassemia disease can include paleness, do not grow as well, and an enlarged spleen.				
Yes	2	18	9	90
No	0	0	1	10
Don't know	9	82	0	0
Thalassemia carriers have no symptoms and look normal.				
Yes	4	36	8	80
No	0	0	2	20
Don't know	7	64	0	0

Table 3. The acceptance of genetic counseling education (n=10)

Questions	#	%
How well did you understand the genetic counseling section?		
It was easy to understand.	7	70
I understood it fairly well.	3	30
Are you able to explain the genetic counseling to others?		
Yes	2	20
Somewhat	8	80
Would you like primary health care staff to use the rabbit charts for client genetic counseling?		
Yes	10	100
No	0	0

Fig 1. Participants' answers about the probability of offspring inheritance for thalassemia (n=10)

A: one parent has disease and one is carrier
B: both parents are carriers
C: one parent is carrier and one is normal



A. B. C.

Table 4. Results of modified one tube osmotic fragility test (OF) and dichlorophenolindophenol precipitate (DCIP) test, hematological analyses for 12 participants.

Partici-pants No.	OF*	DCIP*	Hb (g/dl)	Hct (%)	MCV (pg)	MCH (pg)	MCHC (g/dL)	RDW (%)	Hb Type
1	-	-	15.4	44.2	89.1	31.0	34.8	12.9	A_2A
2	-	-	13.2	37.0	87.6	31.2	35.6	12.6	A_2A
3	+	-	11.9	35.4	88.5	29.7	33.5	14.3	A_2A
4	-	-	12.7	37.4	85.1	29.0	34.1	13.1	A_2A
5	-	-	11.7	33.3	85.5	30.1	35.2	13.4	A_2A
6	-	-	12.3	36.0	87.0	29.7	34.2	13.5	A_2A
7	-	-	12.5	35.7	85.8	30.0	35.0	13.1	A_2A
8	-	-	13.7	37.6	93.0	33.8	36.4	12.5	A_2A
9	-	-	13.0	38.5	88.5	29.9	33.7	13.4	A_2A
10	-	-	12.5	36.0	88.9	30.9	34.8	12.4	A_2A
11	+	-	11.8	35.4	80.9	27.0	33.4	15.8	A_2A
12**	+	+	13.4	39.7	78.8	26.6	33.8	14.4	AE

*Results indicated as positive test (+) or negative test (-)

**Spouse of a Hmong participant who is Laotian

Table 5. Comparison between modified screening results to standard procedure

Participants No.	Modified		Standard	
	OF	DCIP	OF	DCIP
1	-	-	-	-
2	-	-	-	-
3	+	-	+	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	-	-	-	-
8	-	-	-	-
9	-	-	-	-
10	-	-	-	-
11	+	-	+	-
12	+	+	+	+

Table 6. Diagnostic values of OF, DCIP and combined OF and DCIP based on Hb Type. (n=12)

Diagnostic Values (%)	**OF**	**DCIP**	**OF+DCIP**
Sensitivity	100	100	100
Specificity	81.82	100	81.82
+ve Predictive Value	33.33	100	33.33
-ve Predictive Value	100	91.67	100
Accuracy	83.33	91.67	83.33