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Brief communication (Original)

Prevalence of thalassemia carriers among the Lahu hill tribe population, Chiang Rai, Thailand

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Background: Current data on the prevalence of hemoglobinopathies among Lahu in northern Thailand are limited.
Objective: To investigate the prevalence of thalassemia carriers among Lahu hill tribe people in Chiang Rai Province, Thailand.
Materials and Methods: We conducted a cross-sectional study in 2 phases. The first retrospectively analyzed data from antenatal clinics attended by Lahu women between January 2011 and June 2012. The second phase was prospective and included the husbands of Lahu women. In the second phase, 116 Lahu adults were administered a questionnaire and blood tests for osmotic fragility (OFT) and dichlorophenol indophenol precipitation (DCIP). The hemoglobin (Hb) type of those positive for either or both tests was identified by HPLC and PCR.
Results: Data from 358 Lahu women in the first phase showed a mean age of 23.2 y (range 13–46 y, SD 6.83), 68.5% were primigravida. Fifty-eight had abnormal mean corpuscular volume (MCV), 87 positive OFT, 18 positive DCIP test, and 3.0% positive results in both tests. Eight of 83 participating husbands had abnormal MCV, 8 positive OFT and 2 positive DCIP test. In the second phase, 52.2% of 116 participants were women, mean age of 33.7 y (range 18–68 y, SD 11.2), 38 positive OFT, 10 positive DCIP test, and 3.7% positive results in both tests. Hb typing showed 5 participants with HbE, 1 β-thalassemic, 1 HbE homozygous, and no α-thalassemia-1 (SEA) was identified.
Conclusion: Provision of a thalassemia screening in health care settings in remote areas of Thailand is an ongoing need.

Keywords: Hill tribe, Lahu, pregnant women, prevalence, thalassemia carrier

Thalassemia syndromes are the most common genetic hemoglobinopathies worldwide, involving hemoglobin generation in red blood cells, resulting in wide-ranging pathologies of many human organs. Thalassemia and abnormal hemoglobin are common in Southeast Asia [1-3], and are considered causes of the most common chronic hemolytic anemias in Thailand, particularly in northern and northeastern regions [4].

In 2012, Thailand was home to approximately 20 million people carrying genes for thalassemia, which causes numerous signs and symptoms ranging from asymptomatic to severe anemia. Approximately 1% of Thai people or 670,000 have thalassemia, while 40.0% or about 24 million are carriers [5]. Every year, 12,000 children are identified as having thalassemia. Types of thalassemia vary in different regions. In northern Thailand, there are 30.0% with α-thalassemia traits (5.0%-12.0% α-Thal-1, 19.0%-26.0% α-Thal-2), 9.0%-10.0% β-thalassemia, and 8.0% HbE [6].

People with disease forms of thalassemia generally have a low quality of life [7]. The severity has been has quantified by many factors such as age of onset of anemia and age at first transfusion [8]. Thalassemia does not only affect individual health, but also has significant impact on the family, psychosocial, and economic life of patients. The disease needs ongoing long-term and costly treatment, the burden of which is largely borne by the state. In Thailand, demands are particularly high in northern and northeastern regions [9].

So called hill-tribe people of northern Thailand have lived in the mountainous border areas for centuries after migrating there from southern China [10]. The hill tribes are classified into six main groups: Lahu, Akha, Lisu, Karen, Mao, and Yao. In 2013, there

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were almost a million living in the northern region of Thailand and distributing over 7 provinces, with most concentrated in Chiang Rai, Chiang Mai, and Mae Hong Son provinces [11].

The Lahu are the second most populous group (tribe) among hill tribes of Thailand who live in remote mountainous areas. Lahu have their own culture. Their language has been identified as being a Burmese-Lolo branch of Tibeto-Burmese languages [11]. In 2014, there were approximately 711,500 Lahu people in four countries: Thailand, Myanmar, China, and Vietnam with 24 subgroups [11]. Like other hill tribes, many Lahu of Thailand still do not have Thai National Identification Cards that would entitle them to free government health care. Many are illiterate, another barrier for access to free health services and education [12]. In 2012, there were 216 hill-tribe villages with 48,835 people living in Chiang Rai Province [13]. In 2011, Mae Suai Hospital, one of the government hospitals in Chiang Rai Province, reported two cases of stillbirth resulting from severe thalassemia. Both were born to Lahu mothers who never received any antenatal care and lived in very remote areas [14].

Urbanization attracts many Lahu to move out of villages to seek work in cities, mainly in unskilled jobs. Working outside the village increases the opportunity to marry across ethnic lines, which may alter the incidence of thalassemia in and outside their communities. To date, there are no studies of thalassemia among Lahu hill tribes. We therefore investigated thalassemia among Lahu people in Chiang Rai, a province bordering Laos and Myanmar. We hope that the data obtained may be of use for public health planning for intervention needed for thalassemia among this hill-tribe minority population, and also to extend such studies to other tribal groups and locations.

Materials and methods

Study design and study sites

A cross-sectional observational study was used to identify thalassemia carriers among Lahu people who live in Chiang Rai Province. The study consisted of two discrete phases. The first phase statistically and retrospectively analyzed demographic and hematological data from pregnant Lahu women who visited antenatal clinics (ANC) at 4 local hospitals between January 2011 and June 2013. Also studied were their husbands who responded to hospital invitations to have screening tests because their wives had tested positive for possible thalassemia. The second phase of the study was based on collection of similar data from inhabitants of four representative Lahu villages using randomly selected adult participants of both sexes. Hemoglobin (Hb) types in specimens screening positive for thalassemia were identified to indicate the type of thalassemia.

Study samples and research instruments

For the first phase of the study, data were extracted from medical records of all pregnant Lahu women who attended ANC at four local hospitals between January 2011 and June 2013. Data included age, marital status, education, gravidity, height, weight, blood group, osmotic fragility test (OFT) and dichlorophenol indophenol precipitation test (DCIP) test results, which were used as screening tests for thalassemia. Husbands whose wives had positive OFT or DCIP test results were invited to attend the hospital for similar screening. The husbands' results were included in the data analysis of this part of the study.

The second phase consisted of collection of data from 116 adult participants; 29 people from each of the 4 Lahu villages chosen to represent 4 study districts. The districts were: Mae Suai District (61 villages, population 13,626, Lahu people 217), Mae Chan District (18 villages, population 4,393, Lahu people 156), Mae Fah Luang District (49 villages, population 13,080, Lahu people 183), and Wieng Pa Pao District (24 villages, population 6,015, Lahu people 254) [13]. Participant sample sizes were calculated as described below. We used a simple random technique to select representative villages and participants. All participants had 3 mL of blood sent for thalassemia screening tests. Samples with positive screening test results had Hb types identified. None of the participants in the first phase were included in the second phase.

Participants were verbally asked or completed questionnaires with 17 items on general characteristics and socioeconomics, history of anemia, blood transfusion, of self and parents or relatives. The questionnaire had been tested for validity and reliability in a similar group of 10 participants before use.

Sample size estimation

In the first phase, all pregnant Lahu women who visited at least once at any of the 4 ANC clinics between January 2011 and June 2012, were eligible for data analysis including that from their husbands, where the wives had positive test results for either DCIP or OFT, or both.

In the second phase, the sample size of 116 participants was derived from a power calculation based on an a of 5.0%, and 80.0% of the power statistic using Epi-Info, version 6.04 (US Centers for Disease Control and Prevention, Atlanta, GA). Twenty-nine participants were chosen from each of the 4 randomly chosen villages representing 4 districts using a simple random sampling technique.

Data collection

In the first phase, data were extracted from ANC medical records by ANC staff authorized by the hospital directors. To verify that the data was from a Lahu woman we used the patient's name and their residential village name and location to match and check with the database and maps from the district government office, which identify villages where tribal people live almost exclusively. The patient's name provides an important and reasonably reliable clue as to their tribal origin.

In the second phase, information on personal characteristics was obtained in a face-to-face interview. Participants were counseled, and the purpose of this research explained to them. Information on thalassemia was provided before obtaining documented informed consent. Because most participants were illiterate, this consent was witnessed by two health care professionals. After consent 3 mL of blood was obtained. Specimens were coded and transferred to the laboratory of the Chiang Rai Regional Medical Science Center. The interview and blood collection took approximately 30 minutes for each person. Participants were presented with a small gift in appreciation of their participation in the study.

All blood specimens were screened for thalassemia using an OFT and a DCIP test. Those samples with positive results were further analyzed by high performance liquid chromatography (HPLC) for Hb types, and polymerase chain reaction (PCR) for α-thalassemia 1 trait.

Data management and data analysis

Data were double-entered and validated using Microsoft Excel. Data analyses were performed using SPSS (version 11.5, 2006; SPSS, Chicago, IL, USA) and Epi-Info, version 6.04d (US Centers for Disease Control and Prevention, Atlanta, GA, USA). All data were kept secured with password protection accessible only by the researchers. Frequency, percentage, means, and standard deviation (SD) were used to explain the characteristics and prevalence of thalassemia carriers among the participants.

Ethical considerations

Permission to access information in the 4 ANC clinics were granted by the directors of each hospital where the clinics were located. All study forms and procedures were approved by the Committee for the Protection of Human Subjects of Mae Fah Luang University before start of the study (REH-52026). All participants in the second phase received an oral and written explanation and provided their consent before a voluntary agreement was witnessed and documented by signature or fingerprint. The accuracy and understanding of communication with the participants was assured by using local staff who were fluent in both Thai and Lahu languages. The propriety of the small gift presented to the participants to thank them for their participation in the study was considered appropriate in the light of the traditions of the local Lahu culture and would not unduly induce participation.

Results

In phase one, the medical records of 368 pregnant Lahu women were included in the analysis. The results are shown in **Table 1**.

Eighty-three of 87 husbands (95%) of the pregnant Lahu women who had positive OFT and/or DCIP results were tested for OFT and DCIP. The results as shown in **Table 2**.

The general characteristics of the second phase participants were 55% women, 31% of all participants were aged 26–35 years, 82% were buddhist, 82% were employed in agricultural occupations, 64% had no schooling, 16% had chronic diaseses such as diabtes militus or hypertension, and 5% had a history of blood transfusion. Those who had a history of blood transfusion (6 participants) had a severe traffic injury that needed immediate emergency blood transfusion. The 2 cases of relatives and one case of sibling that had a history of blood transfusion had blood transfusions once for unknown diseases, but not because of thalassemia.

Table 3 shows laboratory results of 116 participants from the second phase study. In total, 38 participants (33%) had their Hb were tested by HPLC and PCR. Thalassemia carriers were identified by hemoglobin typing.

Table 1. Osmotic fragility test (OFT) and dichlorophenol indophenol precipitation (DCIP) test results for thalassemia screening of pregnant Lahu women receiving antenatal care between January 2011 and June 2012

Charcteristic	n	%
Total	368	100.00
Age (years)		
≤20	149	40.5
21–30	161	43.8
31–40	50	13.6
≥40	8	2.1
min = 13 y, max = 46 y, mean = 23.23 y, SD = 6.83 y		
Gravidity		
1	162	68.5
≥2	75	31.5
Mean corpuscular volume		
Abnormal (<80 fL)	58	17.6
Normal (80–100 fL)	271	82.4
Hematocrit		
Abnormal (<33%)	94	29.1
Normal (≥33%)	230	70.9
OFT		
Positive	87	23.6
Negative	274	74.5
Unknown		1.9
DCIP test		
Positive	18	5.0
Negative	342	95.0

Table 2. Osmotic fragility test (OFT) and dichlorophenol indophenol precipitation (DCIP) test for thalassemia screening of the husbands of pregnant women who were found positive for either or both of OFT and DCIP tests between January 2011 and June 2012

Charcateristic	n	%
Total	83	100
Age (years)		
≤20	7	19
21–30	20	56
31–40	7	19
≥40	2	6
MCV		
Abnormal (<80 fL)	8	17
Normal (80-100 fL)	40	83
OFT		
Positive	8	10
Negative	75	90
DCIP test		
Positive	2	3
Negative	81	97

Table 3. Thalassemia identification by HPLC and PCR techniques

Charcteristic	n	%
Total	116	100
Osmotic fragility test		
Positive	38	33
Negative	78	67
Dichlorophenol indophenol precipitation test		
Positive	10	9
Negative	106	91
Typing total	38	100
A2A (Hb A2: 2.0%–3.1%) (normal type)	31	82
A2A (Hb A2: 4.9%) (b-thalassemia trait)	1	3
EA (HbE trait)	5	13
EE (HbE Homozygous)	1	3
α-Thalassemia 1 (SEA)	0	0

Discussion

One challenge was to identify Lahu using the ANC list. We used specific characteristics of patients' names and the residential locations. The local ANC clinic staff were most helpful in this respect, as most of them have worked in the area for many years and knew the women, their families, and their villages intimately from their ANC contact of providing counseling, health checks, and dispensing medication on a monthly basis. Pregnant Lahu women selected were only those attending the clinics during the one and half years before the starting point of the study.

In the second phase, women were slightly overrepresented at 55%. This was because interviews took place mainly during the day when men were working away from home. The proportion of participants positive for either the DCIP test or OFT, or both, was not different between the 4 representative villages. The selected villages in the study were approximately 20 km from the hospitals over difficult roads, and there were no other health care centers nearby. People in the villages use their own language; only few could speak Thai. In the counseling and provision of information, we used local people as interpreters.

Villagers in the second study had a higher percentage of positive screening tests than that found for the women attending the ANCs. This suggests that people who are thalassemia carries had a greater prevalence among those who lived in Lahu communities, rather than those who attended the ANCs at hospitals in population centers.

In 2002, Thailand was home to approximately 102,287 Lahu people who lived in 7 provinces: Chiang Rai, Chiang Mai, Tak, Mae Hong Son, Lampang, Petchaboon, and Kamphaeng Phet [11]. This implies that in Thailand approximately 2,660 Lahu people harbor the β-thalassemia trait, and 13,500 the Hb E trait. Because the Lahu hill tribe people in Thailand face many barriers to access the health care system, including the costs of transportation from their village to the hospitals [12]. Almost 60.4% of the pregnant hill-tribe women are illiterate [15]. There are 4 Lahu subgroups. It will be of great benefit if we could identify which subgroup has the greatest proportion of thalassemia carriers by using simple methods that meet standard requirements [16], and obtaining information that can be used for future health care service management for this very vulnerable population.

Dhamcharee et al. [17] reported that not every hospital in Thailand is supported by government funds for thalassemia services, and less than 50% of hospitals had well-organized thalassemia programs with team leaders who are physicians. For patients who do not receive appropriate diagnosis and treatment as the disease progresses, complications can be anticipated [18]. This is a burdensome problem that should be anticipated by a modern society, and which, currently, needs more attention directed to such populations as the hill tribes of the remote mountainous border regions of Thailand.

In summary, the overall prevalence of thalassemia carriers in Lahu among those who had a positive test result for OFT or DCIP test, or both, was 18%.

The prevalence of the HbE trait (13%) and Hb homozygous E (3%) were greater than in the general Thai population that live in northern Thailand [19] (11.2% and 0.73% respectively). However, there was less HbE trait among healthy individuals than in the study by Insiripong et al. [20]. Porniammongkol et al. found among 181 hill-tribe students in Chiang Mai found that the prevalence of anemia was 31% using Hb as an indicator. Results showed that 8% of children had the thalassemia trait and 93% of these children were anemic [21]. Yanola et al. studied the prevalence of anemia, iron deficiency, thalassemia, and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency among 265 Karen hill-tribe children in Chiang Mai Province [22]. They found 4.0% β-thalassemia trait, one case of HbE trait, and no α-thalassemia-1 (SEA) or Thai type deletion. This is not consistent with the present study, which found a high proportion of HbE trait (13%) in the Lahu population studied. The prevalence of β-thalassemia trait (3%) among the Lahu hill tribe people was within the range that was reported among the general Thai population (3%-9%) [5].

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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