RESEARCH NOTE

SURVEY OF COUPLES IN UPPER NORTHERN THAILAND (JANUARY - DECEMBER 2019) AT RISK OF HAVING NEWBORNS WITH THALASSEMIA DISEASE

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Abstract. Prevalence of thalassemia (thal) varies among different regions resulting in different proportions of couples at risk of carrying fetuses with thal disease. From January to December 2019, prevalence and genotypes were determined of couples (n = 370) in upper northern Thailand for risk of having newborns with thal diseases (homozygous α^0-thal (Hb Bart's hydrops fetalis), β-thal major and β-thal/Hb E). Based on Hb typing and DNA analysis, allele frequency of α^0-thal--^{SEA}, β-thal and Hb E was 0.034, 0.072 and 0.086, respectively, and the estimated number of newborns with Hb Bart's hydrops fetalis, β-thal major and β-thal/Hb E per 10,000 pregnancies was 12, 52, and 124, indicating for the identified at-risk couples (n = 28) of 5, 3 and 20 newborns, respectively. β^0-thal codon 17 (A>T) and codon 41/42 (-TCTT) mutations were the most common alleles. Homozygous and compound heterozygous β-thal major individuals were also identified. Information from this study can be applied to prevention and control strategies of these severe thalassemias in Thailand and other parts of Southeast Asia.

Keywords: at-risk couple, β-thalassemia major, β-thalassemia/Hb E, Hb Barts's hydrops fetalis, northern Thailand, severe thalassemia disease

INTRODUCTION

Hemoglobinopathies, include thalassemia (thal) syndromes and structural hemoglobin (Hb) variants, inherited Hb disorders causing red cell pathology, are generally spread throughout Southeast Asia, Southern China, Africa, Middle East, and the Mediterranean (Li, 2017). In Thailand, prevalence of α-thal, β-thal and Hb E (HBB:c.79G>A) is 20-30, 3-9% and up to 54%, resulting in three common thalassemia diseases, namely, homozygous α^0-thal (Hb Bart's hydrops fetalis), β-thal major and β-thal/Hb E (Fucharoen and Winichagoon, 2011). Thus, these three thalassemias are

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key targets for prevention and control program in the country (Fucharoen and Winichagoon, 1992). In regions with large populations and limited resources, rapid diagnostic methods, such as modified one-tube osmotic fragility test (OF test) and/or mean corpuscular volume (MCV) and dichlorophenol-indophenol (DCIP) test are used for α- and β-thal and Hb E screening respectively (Kulapongs *et al*, 1976; Nathalang *et al*, 2005; Sirichotiyakul *et al*, 2005). Samples with positive screening results require confirmation by Hb typing and DNA analysis. A mother at risk of having a newborn with thalassemia disease is then referred to a thalassemia counseling service.

Here, prevalence and genotypes in upper northern Thailand of risk couples having a newborn with thalassemia disease was determined from January to December 2019.

MATERIALS AND METHODS

Blood samples

Peripheral blood of pregnant women and their spouses were collected in ethylenediamine tetra-acetic acid (EDTA)-anticoagulant tube (BD Vacutainer™, Franklin Lakes, NJ) from five public hospitals in Chiang Mai, Chiang Rai, Lampang, Lamphun, and Phrae provinces, upper northern Thailand from January to December 2019.

Thalassemia diagnosis

α- and β-Thal screenings were performed using OF test and/or MCV measurement in an automated hematology analyzer (ADVIA 2120i, Siemens Healthcare Diagnostic, Deerfield, IL), and Hb E screening was by a DCIP test (Kulapongs *et al*, 1976). Blood samples of couples with <85% OF and/or MCV <80 fl (Pengkliang *et al*, 1994; Sirichotiyakul *et al*, 2005), or with positive DCIP test results were subjected to thalassemia confirmation assays at the Thalassemia Unit, Associated Medical Sciences-Clinical Service Center, Chiang Mai University, Chiang Mai where samples were kept at 4°C and assayed within three days of arrival. Capillary electrophoresis (CAPILLARYS™ 2; Sebia, Norcross, GA) was employed for Hb analysis. SYBR Green1 quantitative PCR coupled with high-resolution melting analysis (CFX96 Touch System, BioRad, Hercules, CA) using DNA extracted from blood samples with a NucleoSpin® kit (Macherey-Nagel, KG, Duren, Germany) was carried out for detection of α^0-thal--^{SEA} and --^{Thai} type deletions as previously described (Pornprasert *et al*, 2008; Pornprasert *et al*, 2011). Allele-specific (AS) PCR (Old *et al*, 2005) was conducted for diagnosis of both β^0-thal [codon 17 (A>T) (HBB:c.52A>T), codon 41/42 (-TCTT) (HBB:c.126_129delCTTT), codon 71/72 (+A) (HBB:c.216_217insA), and [IVSI-1 (G>T) (HBB:c.92+1G>T)], and β^+-thal [IVSI-5 (G>C) (HBB:c.92+5G>C), IVSII-654 (C>T) (HBB:c.316-197C>T), nt-28 (A>G) (HBB:c.-78A>G), nt-31 (A>G) (HBB:c.-81A>G), and nt-87 (C>A) (HBB:c.-137C>A)] mutations, and Gap-PCR (Prathomtanapong *et al*, 2009) for β^0-thal 3.4 kb deletion.

RESULTS

From January to December 2019, blood samples from 370 pregnant mothers and their respective spouse were tested for Hb types and presence of β-thal and Hb E alleles. Of the 740 samples, percent β-thal and Hb E allele was 14.4 and 17.1, respectively (six individuals were homozygous Hb E) (Table 1). Only 528 individuals (264 couples) were tested for α-thal, resulting in percent α^0-thal--^{SEA}

of 6.8 (Table 1); no α^0-thal--^{Thai} allele was detected. Hardy-Weinberg equation then was used to estimate the probability of offspring with thalassemia disease per 10,000 pregnancies, indicating 52 fetuses with β-thal major and 124 with β-thal/Hb E and 12 fetuses with homozygous α^0-thal--^{SEA} (Hb Bart's hydrops fetalis). Twenty-eight (8%) at-risk couples were identified, with possible 20 newborns with β^0-thal/Hb E, 5 with Hb Bart's hydrops fetalis, 1 with homozygous β^0-thal [IVSI-1 (G>T)], 1 with homozygous β^+-thal [nt-31 (A>G)], and 1 with compound β^0-thal (3.4 kb deletion) and β^+-thal [nt-31 (A>G)] (Table 2).

Table 1
α- and β-Thalassemia allele frequencies among pregnant mothers and spouses (n = 740) in upper northern Thailand (January - December 2019).

Thalassemia genotype	Number of samples	Number of alleles	Allele frequency
α^0-Thal--^{SEA*}	36	36	0.034
β-Thal	107	107	0.072
Hb E	121	127	0.086

*From 264 couples (n = 528).

Table 2
Potential thalassemia diseases in newborns of at-risk mothers (n = 28) in upper northern Thailand (January - December 2019).

Thalassemia genotype	Number of mothers
Hb Bart's hydrops fetalis	
α--^{SEA}/α--^{SEA}	5
Homozygous/compound heterozygous β-thalassemia	
β^{-31}/β^{-31}	1
$\beta^{-31}/\beta^{3.4\ kb\ deletion}$	1
$\beta^{IVSI-1}/\beta^{IVSI-1}$	1
β^0-thalassemia/Hb E	
$\beta^{codon\ 17}/\beta^E$	11
$\beta^{codon\ 41/42}/\beta^E$	5
β^{IVSI-1}/β^E	3
$\beta^{codon\ 71/72}/\beta^E$	1

DISCUSSION

The key targets of thalassemia prevention and control programs, namely, Hb Bart's hydrops fetalis, β-thal major, and β-thal/Hb E, were identified among in 370 couples in the five upper northern provinces of Thailand during January to December 2019. The allele frequency of α^0-thal--^{SEA} and Hb E was in the range of that previously observed in the Thai population (0.015-0.069 and 0.071-0.268 respectively), while the allele frequency of β-thal was higher than that previously reported (0.009-0.030) (Sengchanh *et al*, 2005; Tienthavorn *et al*, 2006), resulting in a higher estimated possible numbers of newborns with homozygous β-thal and β-thal/Hb E per 10,000 pregnancies than those reported previously (9 and 80 newborns respectively) while that of Hb Bart's hydrops fetalis was comparable (2-28 newborns) (Tienthavorn *et al*, 2006). The allele frequency of α^0-thal--^{SEA} in the present study was higher than that reported from Cambodian, Laotian, Vietnamese, and So (in South Laos) population (0.006-0.014) (Hundrieser *et al*, 1988; Sengchanh *et al*, 2005; Carnley *et al*, 2006). These results indicate that α-thal, β-thal, and Hb E carriers as well as complex thalassemia syndromes are still prevalence and need for continuing prevention and control in not only the upper northern but also others regions of Thailand (Chaibunruang *et al*, 2018; Mankhemthong *et al*, 2019; Wong *et al*, 2020).

The risk of couples in the present study of having a newborn with one of three target thalassemia diseases was in agreement with that of a previous study conducted in this region of the country (Wong *et al*, 2006; Wong *et al*, 2020). A previous study in Phramongkutklao Hospital, Bangkok noted homozygous β^0-thal codon 41/42 (-TCTT) and compound heterozygosity of codon 41/42 (-TCTT) with either β^0-thal or severe β^+-thal were the most frequent genotypes responsible for β-thal major (Traivaree *et al*, 2018). β^0-thal codon 41/42 (-TCTT) mutation is common in β^0-thal/Hb E in Thailand and other parts of Southeast Asia (Fucharoen and Winichagoon, 1997; Wong *et al*, 2006; Galanello and Origa, 2010; Traivaree *et al*, 2018; Mankhemthong *et al*, 2019). However, in the present study, β^0-thal codon 17 (A>T), codon 41/42 (-TCTT), IVSI-1 (G>T) and codon 71/72 (+A) were the common causes of β^0-thal/Hb E, consistent with a previous study in cord blood of 11 pregnancies from at-risk mothers in the same region (Pornprasert and Sukunthamala, 2010).

In conclusion, the study revealed 8% of pregnant others in upper northern Thailand were at risk of bearing a fetus with one of the three target thalassemia diseases in Thailand, namely, Hb Bart's hydrops fetalis, β-thal major and β-thal/Hb E. The findings should be of use in the prevention and control of these severe thalassemia diseases in Thailand and other parts of Southeast Asia.

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CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflicts of interest.

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