THALASSEMIA AND HEMOGLOBINOPATHIES IN A GROUP OF ETHNIC MINORITY IN VIETNAM

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ABSTRACT

Background and purpose: *In Vietnam, an ethnically diverse country, it has been documented that the prevalence of thalassemia and hemoglobinopathies vary considerably among ethnic minorities. This study aimed to determine the prevalence of thalassemia and hemoglobinopathies among the Co-Tu, one of the major ethnic minorities in Central of Vietnam.*

Methods: *A cross sectional survey was conducted in Nam Dong mountainous district, a habitant area of the Co-Tu. Altogether 297 reproductive-age women participated. After obtaining informed consent, blood samples were taken. Red blood cell indices and hemoglobin profiles were analyzed using automated analyzers. DNA analysis was performed to identify α-thalassemia.*

Results: *The overall prevalence for thalassemia and hemoglobinopathies was 47.3%. Hemoglobin Constant Spring (Hb CS) was most common with a prevalence of 25.8% (gene frequency = 0.143); the highest prevalence documented to date. The prevalence of other forms was 13.8% (gene frequency = 0.072) for Hb E, 11.1% (gene frequency = 0.057) for α^+-thalassemia, 0.67% (gene frequency = 0.0034) for α-thalassemia, and 0.34% (gene frequency = 0.0017) for Hb Pakse'. None of the participants had α^0-thalassemia.*

Conclusions: *The study demonstrates the highest prevalence of Hb CS that has ever been reported so far; indicating that Hb CS within the region may originate from this ethnic minority. Further study on haplotype analysis will provide additional insight into this matter.*

Keywords: *Thalassemia, Hemoglobinopathies, Hot spot, Hb Constant Spring (CS), Vietnam.*

I. INTRODUCTION

The inherited disorders of hemoglobin are the commonest monogenic diseases. It has been estimated that 7% of the world's population are carriers. They represent a major public health problem in many areas of the world, including south-east Asia countries (1). Southeast Asia consists of ten countries with a total population of about 400 million. The ethnic origins of people living in these countries are diversified. The most prevalent form of thalassemia in this region is Hb E [β26(B8)Glu->Lys, GAG>AAG; HBB: c.78G>C] with frequencies as high as 40.0 to 60.0% along the Thai-Laos-Cambodian border (1-3,11). α-Thalassemia (α-thal) and β-thalassemia (β-thal) are found throughout the region with a wide range of prevalence from 1.0 to 40.0%. Among structural α-globin chain variants, Hemoglobin Constant Spring [Hb CS; α142, Term->Gln, TAA>CAA (α2); HBA2: c.427T>C] is most common, with

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a frequency of up to 5% of some population in Southeast Asia, although the actual prevalence is not known for many countries (4-6). Hb Paksé [α142, Term->Tyr, TAA>TAT (α2); HBA2: c.429A>T] has been reported in Thailand, Laos and Cambodia as well as for some ethnic minorities of Vietnam with an average frequency of 1.0-2.0% (1,6-11). In Thailand where thalassemia syndromes are highly prevalent, the gene frequencies are 20-30% for α-thalassemia, 3-9% for β-thalassemia, up to 52% for hemoglobin E and 3-6% for Hb CS. Combined different mutations are very common resulting in various clinical syndromes with considerable variability in severity (11).

The problems encountered in mapping the abnormal hemoglobin genes are well exemplified in the case of Vietnam, an ethnically diverse country. Although the Vietnamese Kinh makes up 85% of the population, the remaining 15% comprise over 54 minority ethnic groups (10). As the Kinh have settled in the details and coastal plains, the other minorities mainly inhabit the mountainous areas (12).

Hemoglobin Constant Spring is an unstable α-globin variant causing α-thalassemia phenotype. The prevalence of compound heterozygosity for Hb CS and deletional α^+-thalassemia and its characteristics compared with Hb CS heterozygosity have not been reported. Two community-based surveys have been conducted, one for in southern Vietnam (10) and one for in the Central Vietnam (11), so far. That surveys were found that the gene frequencies for thalassemia vary remarkably among ethnic groups (10). Gen frequencies in the region have documented high levels of α and β thalassemia and Hb E in some of these populations (10,11).

Thua Thien Hue Province is located in the North Central Coast region of Vietnam. The province has eight districts and one provincial city, Hue. The population includes three major ethnic groups, Cơ-Tu, Tà-Ôi, and Bru-Vân Kiều. The majority of the ethnic minorities reside in two mountainous districts in the northern part of the province. One study has been carried out in this area which small number of ethnic groups but it was more prevalent and heterozygous thalassemia and hemoglobinopathies. It was extremely noted high prevalence minority group carry the Hb CS mutation (11,13). This study aimed to determine the prevalence and genetic distribution of thalassemias and hemoglobinopathies among ethnic groups in Thua Thien Hue Province, Central Vietnam. The study provides basic information for further implementation of appropriate preventive and control measures for thalassemias in the region.

Data including the performance characteristics of thalassemia screening, prevalence and heterogeneity of thalassemia and hemoglobinopathies, hematological features, and the molecular basis of diseases are presented.

II. MATERIALS AND METHODS

2.1. Population and samples

A community-based survey was conducted in the Nam Dong mountainous district, located 60 km western of the provincial capital Hue city, Thua Thien Hue province in the North Central Coast region of Vietnam. It is relatively poor and contains sizable ethnic minority population. The district has about 25.000 inhabitants, of who most live in the district capital of Khe Tre. The main source of income is agriculture and forestry. More than half of the population is Kinh (Vietnamese majority), who mainly live in and around Khe Tre. About 45% of the population belongs to an ethnic minority group, predominantly the Cơ-tu ethnic minority group. The Cơ-tu ethnic minority living in five communes where were classified as having a very poor economic status by the local authorities *(Figure 1)*.

Ethnic minority women of reproductive age were defined in this setting as aged between 15 and 49 years, which mainly consisted of Cơ-tu ethnic origin. The subjects were chosen by simple randomly selected.

A total of 297 Cơ-tu ethnic women attending at 5 commune health centers were recruited consecutively during June 2012. Demographic data including ethnicity and blood specimens were collected by local health staff. All women were healthy based on physical examination and anthropometric measurement as well as medical history.

After receiving consent, 3 ml of venous blood sample were taken. All blood samples were stored at 4°C and transferred to Hue Central Hospital, Hue city, Vietnam, for determination of hematological parameters. Parts of blood samples were sent on ice to the Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Khon Kaen University, Thailand, for the diagnosis of thalassemia and hemoglobinopathies.

The study protocol was approved by the Scientific Committee of Hue College of Medicine and Pharmacy and the Institution Review Board of Khon Kaen University.

2.2. Laboratory investigations

Hematological parameters were measured using the KX-21 Sysmex automated blood cell counter (Sysmex, Kobe, Japan). These investigations were done within 8 hours after blood collection. Hemoglobin profiles were analyzed using an automated capillary zone electrophoresis instrument, the Capillarys2 (Sebia, Leisse, France). β-thalassemia was diagnosed in individuals with Hb A_2 levels in excess of 4.0% and MCV <80 fl. All β-thal carriers were investigated further for mutations of the β-globin gene using polymerase chain reaction (PCR) based techniques (14).

All samples were tested for two common a^0-thal mutations, the $--^{SEA}$ and $--^{THAI}$ deletions, and two common nondeletional a^+-thal mutations, Hb CS and Hb Paksé. The deletional form of α^+-thal mutation, the 3.7 kb and 4.2 kb deletions, was identified in 297 DNA samples. These investigations were performed using PCR-based techniques as described previously (15-18).

2.3. Statistical analyses

For data analysis the Stata software program, version 12 was used. Percentage and 95% confidence intervals (95% CI) were assessed to determine the prevalence of thalassemia and hemoglobinopathies. Descriptive statistics, mean and standard deviation, were used to describe hematological parameters.

III.RESULTS

Table 1. Thalassemia genotypes among 297 participants

Thalassemia genotype	n	Percent
Heterozygous state		
- Heterozygous Hb CS	60	20.20 (15.78-25.22)
- Heterozygous Hb E	29	9.76 (6.64-13.72)
- Heterozygous a^+-thal	27	9.09 (6.07-12.95)
- Heterozygous b-thal	1	0.34 (0.16-0.61)
- Heterozygous Hb Pakse'	1	0.34 (0.16-0.61)
Homozygous state		
- Homozygous Hb CS	7	2.36 (0.95-4.79)
- Homozygous Hb E	1	0.34 (0.16-0.61)
Compound heterozygous state		
- a^+-thal with Hb CS	2	0.67 (0.4-1.1)
Double heterozygous state		
- Hb E with Hb CS	7	2.36 (0.95-4.79)
- Hb E with a^+-thal	3	1.01 (0.21-2.92)
- b-thal with Hb CS	1	0.34 (0.16-0.61)
Homozygous Hb E with homozygous a^+-thal	1	0.34 (0.16-0.61)
Total	140	47.14 (41.35-52.99)

The result of hemoglobin analysis by Capillarys2 to detect hemoglobin variants and thalassemias in 297 Catu ethnic minority women of reproductive age are shown in table 1.

The table 1 presents the overall prevalence of thalassemias and hemoglobinopathies were very high at 47.14% (95% CI = 41.35-52.99) for the Cơ-tu ethnic minority people. In which, for Hb CS was highest prevalence at 25.93% (95%CI = 21.04-31.30) and was specially observed with Hb CS homozygotes of 7 women at 2.36% (95% CI = 0.95-4.79). Compound α^+-thal with Hb CS and double heterozygous HbE with Hb CS were found 0.67% and 2.36%, respectively. For Hb E was found in 41 women (13.80%). Two women from 41 Hb E carriers were identified to be a homozygotes for Hb E and the other one was homozygous Hb E with homozygous α^+-thal. For prevalence of α^+-thal was 11.11% (95%CI = 7.78-15.25) which heterozygous α^+-thal was 9.09% and two case were compound heterozygous with Hb CS and one case was identified a homozygous HbE with homozygous α^+-thal. For β-thal were found two women carrier, one of with heterozygous and other was heterozygous β-thal with Hb CS.

None of the participants had α^0-thalassemia.

Table 2. Allele frequencies of each thalassemia type among 297 participants

Thalassemia type	No. of allele	Gene frequency	95% CI
Hb CS[a]	84	0.141	0.114 - 0.172
Hb E[a]	43	0.072	0.053 - 0.096
α^+-thal	34	0.057	0.034 - 0.079
β-thal	2	0.003	0.000 - 0.012
Hb Pakse'	1	0.002	0.000 - 0.009

[a] Including individuals with heterozygous and homozygous states.

The results of DNA analysis in 297 participants are shown in Table 2. There was remarkable the highest gene frequencies for Hb CS at 0.141 (95% CI= 0.114 - 0.172), which include 7 Hb CS cases in the homozygous state, 7 cases Hb CS in interaction with Hb E and one case with β-thal heterozygotes. Whereas the gene frequencies for Hb E and α^+-thal were lower, range from 0.072 (95% CI=0.053-0.096) and 0.057 (95% CI=0.034-0.079), respectively. This study was found one woman with heterozygous Hb Paksé, prevalence was 0.3% (gene frequency = 0.002, 95% CI = 0.000-0.009).

Table 3. Hemoglobin profiles and hematological parameters among participants with different genotypes of Hb CS and other forms of thalassemia in comparison to non-thalassemia group

Thalassemia genotype	n	Hb-type	Hb A_2 or Hb E (%)	Hb CS (%)	Rbc ($x10^{12}$/l)	Hb (g/dl)	MCV (fl)	MCH (pg)	RDW (%)
Hb CS genotype									
- Het. Hb CS[a]	60				4.81±0.35	12.5±0.8	85.3±5.3	25.9±1.5	13.5±0.9
- Homo. Hb CS	7				4.24±0.29	10.0±0.8	85.2±8.3	23.7±2.4	16.0±0.5
- Het. Hb CS with het. Hb E	7				4.85±0.37	12.1±1.0	81.7±3.7	24.9±1.4	14.1±0.6
- Het. Hb CS with het.α^+-thal	2				4.85±0.28	10.5±0.8	75.2±1.1	21.6±0.3	14.5±0.3
- Het. Hb CS with β-thal	1				5.33	11.4	70.8	21.4	13.8

Other forms									
Het. Hb E	29				4.92±0.42	12.5±1.1	82.8±6.3	25.4±1.6	14.1 ±1.6
Het. α^+-thal	27				4.61±0.49	12.1±1.4	87.3±6.9	26.2±2.3	14.2±1.9
Het. Hb E with α^+-thal	3				4.77±0.46	11.9±0.6	82.3±4.4	25.0±2.0	13.9±1.1
Homo. Hb E^b (1)	1				5.94	10.8	62.2	18.1	13.8
Homo Hb E with homo α^+-thal	1				5.45	10.1	64.1	18.6	16.9
Het. β-thal (1)	1				6.28	11.5	64.5	18.3	16.2
Het Pakse' (1)	1				4.86	11.1	77.4	22.9	14.5
Non-thalassemia	157				4.46±0.32	12.89±0.9	91.9±5.3	28.9±1.8	13.2±1.0

The table 3 demonstrates and hematological parameters among participants with different kind of genotypes of Hb CS and other forms of thalassemia in comparison to non-thalassemia group. Compared to non thalassemic individuals, all forms of thalassemia and Hb CS had significantly lower MCV and MCH values. The women with all kind of Hb CS were found anemia significantly lower hemoglobin.

IV. DISCUSSION

Types of thalassemia that are mainly prevalent in Southeast Asia are α-thal, β-thal and Hb E (17). Interaction of these forms leads to a variety of thalassemia syndromes, in particular the three severe forms, i.e. homozygous α^0-thal, homozygous β-thal and Hb E-β-thal diseases. These three diseases are of major public health importance for Thailand and Lao PDR (6). Obviously the types of thalassemia and the distribution of the disease in Central Vietnam are dissimilar to the findings from the neighboring countries such as Lao PDR and Thailand (11).

This study about thalassemia and hemoglobinopathies in ethnic minorities within Thua Thien Hue province is the first report for Central Vietnam. The results are representative for the Co-tu ethnic minority women within the area.

The overall prevalence of all forms of thalassemia among the study population was approximately 11.78%. The prevalence of α^+-thal of 11.11% and β-thal of 0.67% is higher than the prevalence of 3.20% for α^+-thal and 1.20% for β-thal at the same area (10) and comparable to prevalence of 9-20% for all forms of α^+-thal and 3-9% for β-thal and 10-53% for Hb E found in neighboring countries such as for the northeast-Thai population (1,2,17,18). Although, the prevalence of α-, β-thal and Hb E has been estimated, and included in the prevention program, the frequency of some particular types such as α^+ thal, Hb CS and Hb Paksé is still not very well known due to the limited availability of DNA technology needed to identify these carriers in routine practice. Interaction of Hb CS or Hb Paksé with α^0-thal could result in a severe form of the Hb H syndrome. Affected families will need appropriate counseling and care (1).

This investigation is the first detecting a carrier of the rare THAI deletion. The homozygous state and its interaction with the SEA deletion can result in Hb Bart's hydrops fetalis. This mutation has been reported in the Thai population with a gene frequency of 0.0003 (1) and also was identified in some areas in Guangdong province of China (20). For Vietnam, this mutation has never been

reported so far. These findings prove that this mutation is spread throughout Southeast Asia, and should be of concern for medical personnel dealing with the prevention and control of thalassemia in affected areas.

The hot spot for Hb E is the Thai-Laos-Cambodian border (2). Geographically, Thua Thien Hue is located close to Thailand and Laos but the prevalence of Hb E trait in Cơ-tu ethnic minority is rather low at 13.8% (Hb E gene frequency of 0.072) in comparison to the findings across the border. While migration and intermarriages between Thais and Laotians are not uncommon, due to similarities in the language and culture, this is not the case for Vietnam on the one hand site and Thai and Laos on the other hand. Geographical vicinity may not be the key factor attributed to the prevalence of this inherited disease. Even the differences found here between the main Vietnamese ethnicity and ethnic minorities indicate a low population mixture between the two parts of the population.

Thalassemia genotype distribution among Cơ-tu ethnic minority is lowering different ethnic groups to what has been reported from South Vietnam (10). But, it is seen that Hb CS is more prevalent and heterogeneous (Table 2), indicating an unequal distribution of thalassemia and hemoglobinopathies among the Thua Thien Hue population, probably due to the fact that intermarriages do happen often. It is noted that approximately one-fourth (25.93%) of the minority group carry Hb CS (allele frequency = 0.141). This is the highest frequency that has ever been reported in the world's population. The Cơ-Tu is local ethnic group living in mountainous areas of Thua Thien Hue (13). Among these minority, inbreeding is quite common, according to provincial authority, rate of inbreeding was 5.1% at 5 communes where blood samples were collected in 2012 (21), that explaining the high frequency of the mutation. Interaction of this mutation with α^0-thal could lead to severe hemoglobin H disease (22,23), posing a burden of the disease within a group. Due to a small sample size, α^0-thal among the minorities was not observed in this investigation. More surveys are required for implementing an appropriate prevention and control program for thalassemia in this specific population.

The literature review is shown that hematological parameters of thalassemia carriers differ significantly from those of normal individuals (24). Hb homozygotes have a moderate hemolytic anemia with splenomegaly, relatively normal red cell indices (4). The Cơ-tu women with all kind of Hb CS were found anemia significantly lower hemoglobin, a marked reduction in MCV and MCH values was observed in all participants with different kind of genotypes of Hb CS and other forms of thalassemia in comparison to non-thalassemia group. Compared to non thalassemic individuals, all forms of thalassemia and Hb CS had significantly lower MCV and MCH values. Furthermore, seven women with Hb CS homozygotes have a mild anemia (Hb=10.03g/dl), lower MCV and MCH value. The finding in the Hb CS homozygotes in this study is different from the literature (4).

The finding confirms the applicability of hemoglobin, MCV and MCH for thalassemia screening in Vietnamese women. Though screening program for thalassemia is not available in Vietnam, it appears that screening approach using Hb, MCV and MCH is very much feasible.

The result of this study showed that this area had been proposed as the Hot Spot of Hemogobin Constant Spring among ethnic minorities in Vietnam. The study demonstrates the highest prevalence of Hb CS that has ever been reported so far; indicating that Hb CS within the region may originate from this ethnic minority. Further study on haplotype analysis will provide additional insight into this matter.

The findings suggested that it was necessary to establish prevention and control programs for thalassemia in Vietnam. Effort should especially be target at women belonging to a minority groups.

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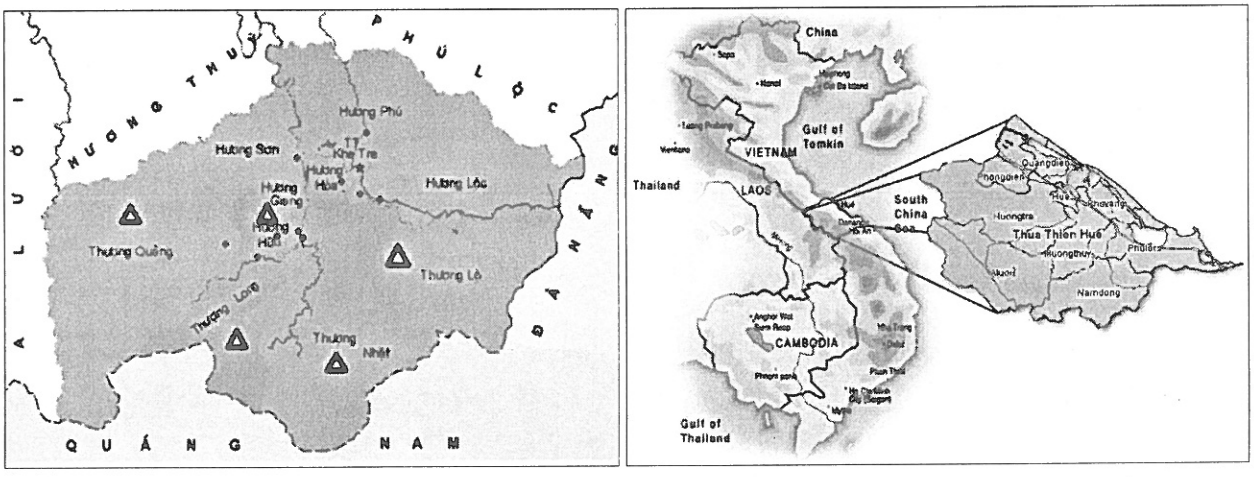

Figure 1: On the left: Location of Nam Dong district, Thua Thien Hue Province in Viet Nam. Right: Map of Nam Dong with open circle where 5 communes were collected samples.