Review Article

Haemoglobinopathies in Southeast Asia

Suthat Fucharoen & Pranee Winichagoon

Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Nakhonpathom, Thailand

In Southeast Asia α-thalassaemia, β-thalassaemia, haemoglobin (Hb) E and Hb Constant Spring (CS) are prevalent. The abnormal genes in different combinations lead to over 60 different thalassaemia syndromes, making Southeast Asia the locality with the most complex thalassaemia genotypes. The four major thalassaemic diseases are Hb Bart's hydrops fetalis (homozygous α-thalassaemia 1), homozygous β-thalassaemia, β-thalassaemia/Hb E and Hb H diseases. α-Thalassaemia, most often, occurs from gene deletions whereas point mutations and small deletions or insertions in the β-globin gene sequence are the major molecular defects responsible for most β-thalassaemias. Clinical manifestations of α-thalassaemia range from asymptomatic cases with normal findings to the totally lethal Hb Bart's hydrops fetalis syndrome. Homozygosity of β-thalassaemia results in a severe thalassaemic disease while the patients with compound heterozygosity, β-thalassaemia/Hb E, present variable severity of anaemia, and some can be as severe as homozygous β-thalassaemia. Concomitant inheritance of α-thalassaemia and increased production of Hb F are responsible for mild clinical phenotypes in some patients. However, there are still some unknown factors that can modulate disease severity in both α- and β-thalassaemias. Therefore, it is possible to set a strategy for prevention and control of thalassaemia, which includes population screening for heterozygotes, genetic counselling and foetal diagnosis with selective abortion of affected pregnancies.

Key words α-thalassaemia - β-thalassaemia - haemoglobin (Hb) E - Hb constant spring (CS)

Introduction

Southeast Asia consists of 10 countries with a total population of about 400 million. The ethnic origins of people living in these countries are very heterogeneous. The Mon-Khmer and Tai language-speaking people occupy Thailand, Laos, Cambodia and some parts of Vietnam, Myanmar and Malaysia. The west includes the Burmese (Tibeto-Burman) and the northeast is the Vietnamese (Austro-Asiatic). The Malayopolynesians (Austronesian) live in Malaysia, Indonesia, Brunei, the Philippines and a number of Pacific island nations. Chinese and Indians are relatively newcomers spread throughout the region[1].

Haemoglobinopathies are the most common genetic disorders among the people living in Southeast Asia. Thalassaemias, globally the commonest monogenic disorders, are a heterogeneous group of anaemias that result from defective synthesis of the globin chains of adult haemoglobin. In Southeast Asia α-thalassaemia, β-thalassaemia, haemoglobin (Hb) E and Hb Constant Spring (CS) are prevalent. The gene frequencies of α-thalassaemia reach 30-40 per cent in Northern Thailand and Laos, 4.5 per cent in Malaysia and 5 per cent in the remote island of the Philippines whereas β-thalassaemias vary between 1 and 9 per cent. Hb E is the hallmark of Southeast Asia attaining a frequency

of 50-60 per cent at the junction of Thailand, Laos, and Cambodia. Hb CS gene frequencies vary between 1 and 8 per cent[2,3]. These abnormal genes in different combinations lead to over 60 different thalassaemia syndromes, making Southeast Asia the locality with the most complex thalassaemia genotypes[4]. The four major thalassaemic diseases are Hb Bart's hydrops fetalis (homozygous α-thalassaemia 1), homozygous β-thalassaemia, β-thalassaemia/Hb E and Hb H diseases.

Clinical manifestations of α-thalassaemia range from asymptomatic cases with normal findings to the totally lethal Hb Bart's hydrops fetalis syndrome. α-Thalassaemia, most often, is due to gene deletions whereas β-thalassaemia is very heterogeneous at the molecular level[5,6]. Point mutations and small deletions or insertions in the β-globin gene sequence are the major molecular defects responsible for most β-thalassaemia. Homozygosity of β-thalassaemia results in a severe thalassaemic disease, in which most patients die within the first decade of life. Compound heterozygosity between β-thalassaemia and Hb E leading to β-thalassaemia/Hb E disease is also common. The patients present variable severity of anaemia, and some can be as severe as homozygous β-thalassaemia[7]. This condition poses a major public health problem throughout Bangladesh, Burma and Southeast Asia. In Sri Lanka nearly 50 per cent of transfusion-dependent thalassaemic children were found to have β-thalassaemia/Hb E[8,9]. In Thailand approximately 3000 new cases are born each year while there may be ten times as many in Indonesia. Therefore, it is timely to set a strategy for prevention and control of thalassaemia, which includes population screening for heterozygote, genetic counselling and foetal diagnosis with selective abortion of affected pregnancies. The national thalassaemia network should be initiated to strengthen the prevention and control programme of thalassaemia in each country.

The α-thalassaemias

α-Thalassaemia is associated with variable degree of α-globin chain deficit that reflects the number of the affected α-globin genes. However, molecular defects of α-thalassaemias are heterogeneous. Normal individuals have two α-globin genes (αα) linked on each chromosome 16. α-Thalassaemia in Southeast Asia is most often due to α-globin gene deletion[10-12]. The severe form of α-thalassaemia, α-thalassaemia 1, involves a deletion that removes interstitially both of the duplicated α-globin genes (--) on one chromosome,

resulting in complete abolishment of α-globin production (α⁰-thalassaemia). Only a few individuals have the phenotypes of α-thalassaemia 1 due to deletions involving the 40 to 33 kb-upstream DNA sequences (HS-40 and -33) that are highly conserved and may contain a remote regulatory element for full α-globin gene expression[13,14].

The milder form of α-thalassaemia, also known as α⁺-thalassaemia, has a reduced α-globin gene expression. This could be caused by either deletional that has one α-globin gene left functioning (-α, previously known as α-thalassaemia 2) or non-deletional mechanism (α^{T}α or αα^T). Two common types of deletional α-thalassaemia 2 have been identified, one involving a deletion of 4.2 kb of DNA (leftward type, -α$^{4.2}$) and the other of 3.7 kb (rightward type, -α$^{3.7}$). The latter is more frequent in Thailand and Southeast Asian countries representing for about 95 per cent of deletional α-thalassaemia 2[11]. The -α$^{4.2}$ deletion is more frequent in Papua New Guinea and Vanuatu in Melanesia. In addition, there are several α-globin chain variants occurred on the chromosomes with the -α$^{3.7}$ or -α$^{4.2}$ type *i.e.* Hb Q-Mahidol (Thailand) in Thai and Chinese, Hb G-Philadelphia in the Black population and Hb J-Tonkarigi in Pacific Island[15-17].

The non-deletional α⁺-thalassaemias are extremely rare and most of the mutations involve the α2 gene, which has a higher expression than the α1 gene with a ratio around 3:1[18]. Haemoglobin Constant Spring (CS), the most common non-deletional α⁺-thalassaemia in Southeast Asia, is a variant with elongated α-globin chains. Hb CS has an α-thalassaemia 2-like effect because the mutation at the termination codon of the α2-globin gene, TAA->CAA, results in an unstable mRNA and only small amounts of Hb CS are produced[19]. However, it has been found that about 15 per cent of patients previously reported to be Hb CS by haemoglobin analysis are in fact Hb Pakse, TAA->TAT in α2 codon 142[20]. Other non-deletional α⁺-thalassaemias occurring from the highly unstable α-globin chains include Hb Suan-Dok (CTG->CGG in α2 codon 109), Hb Quong Sze (CTG->CCG in α2 codon 125), Hb Pak Num Po (+T in α1 codon 131/132) and Hb Adana (GGC->GAC in α2 codon 59)[21].

Generally haematologic examination in the adult is not reliable for definite diagnosis of α-thalassaemia heterozygote. This is because α-thalassaemia 2 heterozygote is often indistinguishable from normal, and red blood cells of α-thalassaemia 1 heterozygote or α-thalassaemia 2 homozygote have microcytosis, which also occur in iron deficiency anaemia. However,

a deficiency of α-globin chains leads to the excess γ-globins that form γ_4 tetramers (Hb Bart's) in the foetus, and the amount of Hb Bart's in the cord blood may be used to predict the genotype of α-thalassaemia.

% Hb Bart's	Genotypes
1-2	α-thalassaemia 2 heterozygote
5-8	α-thalassaemia 1 heterozygote or α-thalassaemia 2 homozygote
25	Hb H disease
80-85	Hb Bart's hydrops fetalis

Accurate diagnosis for α-thalassaemia syndromes is achieved by molecular biology techniques, mostly related to polymerase chain reaction (PCR).

Clinical heterogeneity of α-thalassaemia

In general, the clinical severity of α-thalassaemia phenotype relates to the number of affected α-globin genes. Individuals who have a deletion or inactivation of one α-globin gene usually do not present significant haematologic changes. When two α-globin genes are deleted either on the same chromosome (α-thalassaemia 1 heterozygote) or one on each chromosome (α-thalassaemia 2 homozygote), hypochromic and microcytic red blood cells are observed without anaemia. Inactivation of 3 α-globin genes due to deletions with or without non-deletional α-thalassaemia leads to the thalassaemia intermedia, called Hb H disease. Whereas the deletion of all four α-globin genes results in Hb Bart's hydrops fetalis, the most severe and lethal thalassaemia disease.

Haemoglobin Bart's hydrops fetalis

Homozygous α-thalassaemia 1 is also known as Hb Bart's hydrops fetalis due to the presence of tetrameric unbound γ-like foetal globin chains (γ_4, Hb Bart's). Although different forms of α-thalassaemia alleles have a worldwide distribution, the occurrence of Hb Bart's hydrops fetalis is almost confined to this region where the highest incidence of α-thalassaemia 1 exists[3]. Because of the absence of α-globin chain synthesis, the foetus does not have either Hb F or Hb A. Haemoglobin electrophoresis shows large amounts of Hb Bart's and about 10-15 per cent Hb Portland ($\zeta_2\gamma_2$). The foetus dies *in utero* or soon after birth because Hb Bart's does not release O_2 to the tissues. The affected foetus is hydropic with abnormal development of vital organs such as brain and lung, and is thus incompatible with life. Maternal complications such as toxaemia of pregnancy have been observed in almost all pregnancies (Vaeusorn, personal communication). Ultrasonography

provides unambiguous detection of Hb Bart's hydrops fetalis at 18-20 wk of pregnancy[22]. DNA diagnosis from chorionic villi or amniotic fluid fibroblasts can detect Hb Bart's hydrops fetalis as early as 10-16 wk gestation[23]. Therapeutic abortion is suggested for the foetus diagnosed as having this disease.

Hb H disease

Usually the patients have no symptoms and do not need treatment when they are in a steady state. Correct diagnosis and understanding of the clinical phenotype are important; otherwise it causes anxiety and mishandling. In general, physical development of the patient is normal and only mild anaemia and jaundice are noticeable. However, the patients may have a sudden fall of haemoglobin levels with severe anaemia and jaundice after acute infection. Proper management of haemolytic crisis is important. Blood transfusion should be given along with treatment for infection and body temperature should be normalized as quickly as possible to reduce induction of Hb H precipitation occurring as the result of fever[24].

Although most of Hb H patients are thalassaemia intermedia, there is a markedly phenotypic variability and the patients with identical α-globin genotypes can have different phenotypes. Two common genotypes lead to the occurrence of Hb H disease in Southeast Asia, *i.e.* α-thalassaemia 1/α-thalassaemia 2 and α-thalassaemia 1/Hb CS[25,26]. Both genotypes have similar clinical manifestations although the latter is slightly more anaemic and may have significant hepatosplenomegaly with requirement of transfusion. The average haemoglobin is 100 ± 12 g/l in α-thalassaemia 1/α-thalassaemia 2 and 96 ± 11 g/l in α-thalassaemia 1/Hb CS. This is because the expression of the two α-globin genes, α1 and α2, are unequal and the α2-globin mRNA is produced at a higher level than the α1-globin mRNA; the abnormality on the α2-globin gene will result in a more defective α-thalassaemia. This is evident by the fact that homozygosity or compound heterozygosity of non-deletional α^+-thalassaemias involving the α2-globin gene can lead to a phenotype similar to Hb H disease. These include Hb CS/Hb CS, Hb CS/Hb Pakse, Hb CS/Hb Adana. Furthermore the latter, Hb Adana (Gly->Asp in α2 codon 59), which is a highly unstable haemoglobin variant, in combination with α-thalassaemia 1 can lead to the most severe form of Hb H disease called Hb H hydrops fetalis[27,28]. The foetus suffers from severe anaemia, hypoxia, and consequently with oedema, congenital abnormalities and death. Its

interaction with other α-thalassaemia mutations also contributes to phenotypic variability of Hb H disease in Indonesian patients (Iswari Setianningsih, personel communication).

At birth the umbilical cord blood of the babies with Hb H disease contains about 25 per cent Hb Bart's. In adults the excess β-globin chains form β_4 tetramers (Hb H), thus haemoglobin type is A+H. Hb H levels are usually higher in non-deletional Hb H disease than in the deletional genotype, 12.1±5.5 vs 7.9±4.2 per cent, respectively[29]. Hb H is relatively unstable and can be oxidized to form intracellular precipitates. Multiple intraerythrocytic inclusion bodies can be detected by mixing a drop of blood with methylene blue or Brilliant Cresyl Blue. They are also higher in non-deletional Hb H disease, 77±10 vs 66±11 per cent. Formation of intraerythrocytic inclusion bodies was enhanced when the patients have fever and this might cause acute haemolytic crisis and suddenly severe anaemia.

Hb AE Bart's and Hb EF Bart's diseases

This is the result of complex gene interactions of α- and β-thalassaemias. They present with thalassaemia intermedia phenotype and the symptoms are usually similar to Hb H disease. Hb AEBart's disease occurs as a result of inheritance of three specific genes, α-thalassaemia 1/α-thalassaemia 2 + Hb E or α-thalassaemia 1/Hb CS + Hb E. The typical characteristic of this disease is the haemoglobin phenotype of A+E+Bart's or CS+A+E+Bart's, respectively. Hb E constitutes 13-15 per cent of total haemoglobin because of the lower affinity of the β-globin chains for β^E-globin chains as compared with the normal β-globin chains. Approximately 5-6 per cent of RBC can be demonstrated to contain Hb H inclusions, although Hb H is too low to be demonstrated by haemoglobin electrophoresis[30].

EF Bart's disease is an uncommon form of thalassaemia intermedia resulting from the co-inheritance of the abnormal α- and β-globin genes. Haemoglobin electrophoresis revealed the typical phenotypes of E+F+Bart's or CS+E+F+Bart's. Hb E is the major component constituting about 85 per cent, with the minor components of Hb F and Bart's. Haemoglobin levels ranged between 61 and 104 g/l. DNA analysis indicates that four genotypes involving 5 abnormal globin genes are responsible for this thalassaemia syndrome[31]. These are: Hb H (α-thalassaemia 1/α-thalassaemia 2) and homozygous Hb E; Hb H (α-thalassaemia 1/α-thalassaemia 2)

and β-thalassaemia/Hb E; Hb H-CS (α-thalassaemia 1/Hb CS) and homozygous Hb E; and Hb H-CS (α-thalassaemia 1/Hb CS) and β-thalassaemia/Hb E.

Among these, Hb H-CS (α-thalassaemia 1/Hb CS) and homozygous Hb E patients are most common. A possible reason is that the presence of Hb CS leads to a more severe thalassaemia disease as is found in Hb H-CS disease.

The β-thalassaemias

β-Thalassaemias are very heterogeneous, both in the molecular defects and the clinical manifestations. In Southeast Asia β^0-thalassaemia far exceeds β^+-thalassaemia. Different molecular mechanisms, most of which are base substitutions or small deletions or insertions of one or two nucleotides in the β-globin gene are responsible for β-thalassaemia[6,32]. Moreover, it has been found that β-thalassaemia mutations are relatively population specific, i.e. each ethnic group has its own set of common mutants[33-36]. Haemoglobin E occurs from a mutation at position 26 of the β-globin chain (glu->lys). The abnormal gene also results in the reduced amounts of β^E-mRNA and in synthesis of β^E-globin chains[37]. Therefore, Hb E has a mild β^+-thalassaemia phenotype and homozygous Hb E is asymptomatic. There is no evidence of anaemia and jaundice, and hepatosplenomegaly is not observed in the majority of cases. Globally, by far the most important intermediate form of β-thalassaemia is β-thalassaemia/Hb E, which is much more common than homozygous β-thalassaemia. Furthermore, in every country in which β-thalassaemia is common, clinically similar intermediate forms also constitute with increasing proportion of the clinical load imposed by the disease.

Homozygous β-thalassaemia is a severe disease. Children with β-thalassaemia major appear healthy at birth because Hb F is still functioning. Pathology occurs when the α-globin gene is switched off at 8 to 12 months after birth but there is no β-globin expression. Defects in the globin genes result in impaired globin chain synthesis leading to the reduced haemoglobin content of red cells and consequent red cell pathology. Ineffective erythropoiesis and shortened red cell survival will lead to chronic anaemia in thalassaemia. Without treatment, the spleen, liver, and heart become greatly enlarged. During the first decade of their life they become pale, listless and fussy, and have a poor appetite. They grow slowly and often develop jaundice. Bones turn thin and brittle, osteoporosis and osteopenia, and

face bones become distorted. In this region many children with major thalassaemic diseases are still treated by a minimal transfusion programme and only a few of them receive regular iron chelation. Most of these thalassaemia major patients die in the paediatric age group. Heart failure due to anaemia and infection are the leading causes of death among untreated children.

β-Thalassaemia/Hb E has a wide spectrum of severity. The haemoglobin levels range between 30 and 130 g/l[7,38,39]. The patients with a very low haemoglobin levels have defective physical development, thalassaemic facies, jaundice and hepatosplenomegaly similar to homozygous β-thalassaemia cases. Excess α-globin chains cause ineffective erythropoiesis that leads to anaemia and increased haemolysis leads to jaundice. Severe anaemia results in massive erythropoiesis with several consequences such as defective physical development and thalassaemic facies resulting from the expansion of the bone cavity. Extramedullary haemopoiesis leads to enlarged liver and spleen. The massive erythropoiesis also induces increased iron absorption from the intestine which results in iron overload[40-42]. Degenerative changes followed iron overload can cause cardiac failure, hepatic fibrosis, and other complications as occurs in other thalassaemia major cases. Severe thalassaemic patients may develop hypersplenism in which splenectomy must be considered. However, many complications are observed among splenectomized cases. Severe infections are major causes of death in splenectomized patients. The causative organisms may differ in children and adult patients and the pattern of infections may differ among different populations. Splenectomized patients have frequently been found to have low arterial oxygen saturation or hypoxaemia. Thromboembolism in the pulmonary arteries is frequently detected in adult patients at autopsy. Hypoxaemia may be related to platelet aggregation in the pulmonary circulation and treatment with platelet aggregation inhibitors such as aspirin is beneficial to patients with reversible hypoxaemia[43,44].

The clinical picture of the compound heterozygous state for β-thalassaemia and Hb E can be influenced by both genetic and environmental factors. Well defined genetic modifiers include different interactions of β-thalassaemia alleles of varying severity, co-inheritance of α-thalassaemia alleles, and the relative level of persistent foetal haemoglobin synthesis.

The latter appears to be the most important factor in phenotypic modification.

Modifying factors of severity in β-thalassaemia/Hb E disease

To identify genetic modifiers influencing disease severity, we have conducted a genome-wide association study in a group of Thai β-thalassaemia/ HbE patients. The clinically mild and severe β-thalassaemia/Hb E patients were classified based on their disease severities, after exclusion of α- and β+-thalassaemia co-inheritances, using a validated scoring system[45]. Six representative parameters included haemoglobin level, age of onset, age at first blood transfusion, requirement of blood transfusion, spleen size or splenectomy and growth. Twenty three single nucleotide polymorphisms (SNPs) in 3 independent genes/regions were identified to be significantly associated with the disease severity. The highest association was observed with SNPs on the β-globin gene cluster (chr.11p15). The others were the intergenic region between the *HBS1L* and *MYB* genes (chr.6q23) and the *BCL11A* gene (chr.2p16.1)[46]. All three loci were replicated in an independent cohort of Indonesian patients. In addition, association to the foetal haemoglobin level, which is one of the major modifying factors of severity in β-thalassaemia/Hb E disease was also observed with SNPs on these three regions.

In the β-globin gene cluster linkage disequilibrium (LD) analysis revealed a large LD block spanning from a locus control region (LCR) to the δ-globin gene, which includes the four functional β-globin-like genes (*HBE1, HBG2, HBG1* and *HBD* genes) and one pseudogene (*HBBP1*, haemoglobin beta pseudogene 1). The most significant association to disease severity is the SNP located on *HBBP1* gene, which is surrounded by two functional genes, *HBG1* and *HBD*. Our study[47,48] also showed that this SNP was highly linked with the *Xmn*I polymorphism 158 bp upstream to the *HBG2* gene previously reported to be associated with HbF levels. We hypothesized that these may act as co-regulating to affect the HbF level.

The second most significant region was mapped on the *HBS1L-MYB* intergenic region on chromosome 6q23. The most significant SNP in this region is in strong linkage disequilibrium with previously reported SNPs that also showed a significant association with F cell levels[49]. The third significant gene is the *BCL11A* on chromosome 2p15. The most significant SNP of this region is located on intron 2 of *BCL11A* gene. This

SNP is located in the same LD block to SNP that has been reported as the most highly-associated SNP with Hb F levels[50,51]. Nevertheless, until now the biological effects of these polymorphisms in modulating levels of Hb F are still unknown. Hence our study confirmed the evidence of an association among the β-globin gene cluster, *HBS1L-MYB,* and *BCL11A* that act as the modifying factors for β-thalassaemia severity.

Previous studies have shown that *Xmn1* accounts for approximately 13 per cent of the variation in Hb F in normal Europeans and for a similar proportion in a Chinese sample heterozygous for the β-thalassaemia mutation[52]. In addition, about 15 per cent of the Hb F variance was explained by a polymorphism in *BCL11A* on 2p15, 19 per cent by an intergenic variant in *HBS1L-MYB* on 6q23, and 10 per cent by *Xmn1* in Europeans without haemoglobinopathies. Increased foetal haemoglobin levels can ameliorate the disease severity of β-thalassaemia by reducing imbalanced globin chain synthesis. Nevertheless, the Hb F level alone cannot fully explain the variation of disease severity in β^0-thalassaemia/Hb E disease. In order to investigate the epistasis among these three regions to Hb F levels, the three most highly-associated SNPs with Hb F levels in each locus were selected and the box plots of foetal haemoglobin levels with the numbers of high-Hb F allele(s) were performed. The analysis showed the increased Hb F levels according to the increased numbers of high-Hb F allele(s)[46]. The frequency of high-Hb F alleles in each SNP may be different among populations. The most significant SNP associated with disease severity in Thai population may not be the best SNP that represented as the best marker to predict the disease severity in the other population. Therefore, genotyping of the additional SNPs on the three loci are required to find the most informative SNPs for prediction of disease severity in other populations.

Prevention and control of thalassaemia

Prevention and control of thalassaemias in the entire population of any country requires a well planned programme to establish the epidemiology of the disorders and education to raise the awareness of genetic risk in the medical professionals and the population at large. Two strategic plans should be considered. First is to offer the best treatment to improve the patients' quality of life, the best possible management should be made available to the affected patients. The basic treatment for thalassaemia including blood transfusion and iron chelation should be given to all severe cases. Second is to prevent the birth of

new cases with thalassaemia diseases, which includes carrier screening, genetic counselling and prenatal diagnosis for the couples at risk. This approach may be by population screening or screening at prenatal clinics. When the women are found to be carriers, their partners should be screened. Prevention of further thalassemic offspring in the case of couples at risk can be planned by prenatal diagnosis and selective abortion. Accurate diagnosis and understanding of gene-gene interaction are important for genetic counselling and for the success of the prevention and control of thalassaemias.

This approach is cost-effective and is proving remarkably successful in reducing the frequency of thalassaemia in many countries[53-56]. In Thailand it has been estimated that the total cost for treating four major thalassaemic diseases namely Hb Bart's hydrops fetalis, homozygous β-thalassaemia, β-thalassaemia/Hb E and Hb H diseasesis is about US$ 220 million per year[57]. But elsewhere the cost of a total prevention programme has been demonstrated to be 1/5th to 1/10th of the cost of treating the existing affected patients[58]. In Thailand more than 85 per cent of all pregnant women, the target population for primary screening, are screened annually. A pilot study in the North, at Faculty of Medicine Chiangmai University, where thalassaaemia is most prevalent showed abrupt reduction of all new cases of severe thalassaemia diseases[59]. Several medical centers in other countries including those in the Mediterranean region, China, India, Lebanon, Pakistan and Iran are running the prospective programme for the prevention of thalassaemia. This will result in a reduction of newborns with serious forms of thalassaemia, Hb Bart's hydrops fetalis, homozygous β-thalassaemia and β-thalassaemia/Hb E diseases.

Laboratory diagnosis and screening test for thalassaemia

The conventional diagnosis for thalassaemia carriers is usually based on measurement of the low mean red cell volume (MCV) and mean red cell haemoglobin (MCH) and the haemoglobin typing. Diagnosis of β-thalassaemia carrier is further confirmed by raised level of Hb A_2 determined by the automated HPLC (high-performance liquid chromatography) or capillary electrophoresis (CE)[60]. However, these equipment are expensive resulting in an uneconomical high cost per sample. Therefore, simple screening with combination of tests including the one tube osmotic fragility or MCV/MCH, the DCIP (dichlorophenolindophenol) dye test to detect unstable Hb E, and the plasma ferritin screening test was

developed[61-64]. In Southeast Asia, both thalassaemias with iron overload and patients with iron deficiency co-exist in the same population. Thalassaemias with iron overload need iron chelation while those with iron deficiency require iron supplementation or fortification. If the two conditions are not clearly discriminated the iron-overloaded thalassaemics will be adversely affected.

The screening by one tube osmotic fragility test using 0.36 per cent buffered saline is sensitive enough to detect almost all cases of α-thalassaemia 1 and β-thalassaemia trait while over 96 per cent of normal red cells are haemolysed[62]. The red cells of patients with thalassaemia and iron deficiency anaemia are decreased in osmotic fragility. However, in the vicinity of 100 g/l haemoglobin level the red cell osmotic fragility in most cases of iron deficiency anaemia is normal or slightly decreased.

The blue dye DCIP can be used to detect unstable haemoglobin, including Hb E which lacks stability because the weak α1β1 contact. Hb E molecules dissociate and precipitate upon incubation with the dye at 37°C. No precipitation or cloudy appearance is observed in normal, iron deficiency anaemia and thalassaemia trait subjects[61].

Treatment

The mainstay of treatment for thalassaemia major is regular blood transfusion to maintain adequate levels of the haemoglobin concentration that results in good quality of life. Iron overload in beta-thalassaemia patients is secondary to either multiple blood transfusions or increased iron absorption or the combination of both. Due to the large number of patients and limited medical resources it is not possible to give optimal blood transfusions to the majority of patients. They received no or minimal blood transfusions and no iron chelator such as desferrioxamine which is very expensive. However, the patients developed certain degree of iron overload when they come to the 3rd or 4th decades of life even with minimal blood transfusions. These masses of untreated thalassaemic patients develop multitude of complications such as infections, hypoxaemia, pulmonary hypertension, immunologic abnormalities and trace metals disturbances. In the absence of iron chelation, death from iron-induced heart failure occurs by the mid-teenage years. Oral iron chelator is now available, however, it is still very expensive or need special monitor. The only cure for thalassaemia by stem cell transplantation is available in some countries only. It has been shown that it is more cost-effective to provide stem cell transplantation for the thalassaemia before the age of 10, however, there is a problem to find appropriate HLA match donor.

Conclusion

With its high prevalence thalassaemia is causing an increasingly severe public health burden for many countries in Southeast Asia. Since thalassaemia carriers have no symptoms and because of the complexity and heterogeneity of the disease it is difficult to diagnose thalassaemia, especially the carriers, at the small health care units. Moreover, treatment of severe thalassaemia with regular blood transfusion and iron chelation is expensive. Thus, it is difficult to persuade international health agencies and governments that thalassaemia is a genuine problem of public health importance compared with other communicable diseases that these countries are handling. WHO report adopted by the 57th World Health Assembly in 2004, Genomics and World Health, recommend that both North/South and local networks should be established as an aid to helping member countries to evolve these services[65]. During the last few years a cohesive group of professional staff including the haematologists and geneticists of many countries in Asia joined hands together as Asian Network for Thalassaemia Control to disseminate good practice in the control and management of thalassaemia in Asia and to provide a central forum for interacting with individual governments and international health agencies to provide support for this objective. The main objectives are as follows: *(i)* the development and dissemination of adequate screening techniques to determine the frequency of the different forms of thalassaemia in Asian countries, *(ii)* further evolution of education and screening programmes and discussions about the possibility of prenatal diagnosis as an interim method for the control of thalassaemia, and *(iii)* the development of more adequate approaches to treatment. It is expected that within the next few years, we can help many countries in Asia to develop the national programme for the prevention and control of thalassaemia.

References

1.   Abdulla MA, Ahmed I, Assawamakin A, Bhak J, Brahmachari SK, Calacal GC, *et al*. Mapping human genetic diversity in Asia. *Science* 2009; *326* : 1541-5.

2. Wasi P. Hemoglobinopathies in Southeast Asia. In: Bowman JE, editor. *Distribution and evolution of hemoglobin and globin Loci*. New York: Elsevier; 1983. p. 179-203.

3. Fucharoen S, Winichagoon P. Hemoglobinopathies in Southeast Asia. *Hemoglobin* 1987; *11* : 65-8.

4. Wasi P, Na Nakorn S, Pootrakul S, Sookanek M, Disthansongchan P, Panich V, *et al*. Alpha- and beta-thalassemia in Thailand. *Ann NY Acad Sci* 1969; *165* : 60-82.

5. Higgs DR, Vickers MA, Wilkie AOM, Pretorius IM, Jarman AP, Weatherall DJ. A review of the molecular genetics of the human α-globin gene cluster. *Blood* 1989; *73* : 1081-104.

6. Thein SL, Winichagoon P, Hesketh C, Best S, Fucharoen S, Wasi P, *et al*. The Molecular basis of β-thalassemia in Thailand: Application to prenatal diagnosis. *Am J Hum Genet* 1990; *47* : 369-75.

7. Fucharoen S, Winichagoon P, Pootrakul P, Piankijagum A, Wasi P. Variable severity of Southeast Asian β-thalassemia/Hb E disease. *Birth Defects* 1988; *23* (SA): 241-8.

8. Olivieri NF, Thayalsuthan V, O'Donnell A, Premawardhena A, Rigobon C, Muraca G, *et al*. Emerging insights in the management of hemoglobin E beta thalassemia. *Ann NY Acad Sci* 2010; *1202* : 155-7.

9. Premawardhena A, Fisher CA, Olivieri NF, de Silva S, Arambepola M, Perera W, *et al*. Haemoglobin E thalassaemia in Sri Lanka. *Lancet* 2005; *366* : 1467-70.

10. Lie Injo LE, Solai A, Herrera AR, Nicolaisen L, Kan YW, Wan WP, *et al*. Hb Bart's level in cord blood and deletion of α-globin genes. *Blood* 1982; *59* : 370-6.

11. Winichagoon P, Higgs DR, Goodbourn SEY, Clegg JB, Weatherall DJ, Wasi P. The molecular basis of α-thalassemia in Thailand. *EMBO J* 1984; *3* : 1813-8.

12. Fischel-Ghodsian N, Vickers MA, Seip M, Winichagoon P, Higgs DR. Characterization of two deletions that remove the entire human α-globin gene complex (--THAI and --FIL). *Br J Haematol* 1988; *70* : 233-8.

13. Higgs DR, Garrick D, Anguita E, De Gobbi M, Hughes J, Muers M, *et al*. Understanding alpha-globin gene regulation: aiming to improve the management of thalassemia. *Ann NY Acad Sci* 2005; *1054* : 92-102.

14. Viprakasit V, Harteveld CL, Ayyub H, Stanley JS, Giordano PC, Wood WG, *et al*. A novel deletion causing alpha thalassemia clarifies the importance of the major human alpha globin regulatory element. *Blood* 2006; *107* : 3811-2.

15. Flint J, Harding RM, Boyce AJ, Clegg JB. The population genetics of the haemoglobinopathies. *Baillieres Clin Haematol* 1998; *11* : 1-51.

16. Weatherall DJ, Clegg JB, editors. *The thassaemia syndromes*. 4th ed. Oxford, United Kingdom: Blackwell Science; 2001.

17. Weatherall DJ, Miller LH, Baruch DI, Marsh K, Doumbo OK, Casals-Pascual C, *et al*. Malaria and the red cell. *Hematology Am Soc Hematol Educ Program 2002* : 35-57.

18. Liebhaber SA, Cash FE, Ballas SK. Human α-globin gene expression. The dominant role of the α2-locus in mRNA and protein synthesis. *J Biol Chem* 1986; *261* : 15327-33.

19. Liebhaber SA, Cash F, Eshleman SS. Translation inhibition by an mRNA coding region secondary structure is determined by its proximity to the AUG initiation codon. *J Mol Biol* 1992; *226* : 609-21.

20. Viprakasit V, Tanphaichitr VS, Pung-Amritt P, Petrarat S, Suwantol L, Fisher C, *et al*. Clinical phenotypes and molecular characterization of Hb H-Pakse disease. *Haematologica* 2002; *87* : 117-25.

21. Viprakasit V, Tanphaichitr VS, Veerakul G, Chinchang W, Petrarat S, Pung-Amritt P, *et al*. Co-inheritance of Hb Pak Num Po, a novel alpha1 gene mutation, and alpha0 thalassemia associated with transfusion-dependent Hb H disease. *Am J Hematol* 2004; *75* : 157-63.

22. Kanokpongsakdi S, Fucharoen S, Vantanasiri C, Thonglairoam V, Winichagoon P, Manassakorn J. Ultrasonographic method for detection of haemoglobin Bart's hydrops fetalis in the second trimester of pregnancy. *Prenatal Diagnosis* 1990; *10* : 809-13.

23. Fucharoen S, Winichagoon P, Thonglairoam V, Siriboon W, Siritanaratkul N, Kanokpongsakdi S, *et al*. Prenatal diagnosis of thalassemia and hemoglobinopathies in Thailand: Experience from 100 pregnancies. *Southeast Asian J Trop Med Public Health* 1991; *22* : 16-29.

24. Chinprasertsuk S, Piankijagum A, Wasi P. *In vivo* induction of intraerythrocytic inclusion bodies in hemoglobin H disease: an electron microscopic study. *Birth Defects* 1988; *23* : 317-26.

25. Fucharoen S, Winichagoon P, Pootrakul P, Piankijagum A, Wasi P. Differences between two types of Hb H disease, α-thalassemia 1/α-thalassemia 2 and α-thalassemia 1/Hb Constant Spring. *Birth Defects* 1988; *23* : 309-15.

26. Chan V, Chan TK, Todd D. Different forms of Hb H disease in the Chinese. *Hemoglobin* 1988; *12* : 499-507.

27. Chui DH, Fucharoen S, Chan V. Hemoglobin H disease: not necessarily a benign disorder. *Blood* 2003; *101* : 791-800.

28. Fucharoen S, Viprakasit V. Hb H disease : clinical course and disease modifiers. *Hematology Am Soc Hematol Educ Program* 2009; 26-34.

29. Winichagoon P, Adirojnanon P, Wasi P. Levels of haemoglobin H and proportions of red cells with inclusion bodies in the two types of haemoglobin H disease. *Br J Haematol* 1980; *46* : 507-9.

30. Thonglairoam V, Winichagoon P, Fucharoen S, Tanphaichitr VS, Pung-Amritt P, Embury SH, *et al*. Hemoglobin Constant Spring in Bangkok: Molecular screening by selective enzymatic amplification of the α2-globin gene. *Am J Hematol* 1991; *38* : 277-80.

31. Fucharoen S, Winichagoon P, Thonglairoam V, Wasi P. EF Bart's disease: interaction of the abnormal α- and β-globin genes. *Eur J Haematol* 1988; *40* : 75-8.

32. Winichagoon P, Fucharoen S, Thonglairoam V, Tanapotiwirut V, Wasi P. β-thalassemia in Thailand. *Ann NY Acad Sci* 1990; *612* : 31-42.

33. Furuumi H, Firdous N, Inoue T, Ohta H, Winichagoon P, Fucharoen S, *et al*. Molecular basis of β-thalassemia in the Maldives. *Hemoglobin* 1998; *22* : 141-51.

34. Setianingsih I, Williamson R, Dand D, Harahap A, Marzuki S, Forrest S. Phenotypic variability of Filipino β(0)-thalassemia/ HbE patients in Indonesia. *Am J Hematol* 1999; *62* : 7-12.

35. Colah R, Gorakshakar A, Nadkarni A, Phanasgaonkar S, Surve R, Sawant P, *et al*. Regional heterogeneity of β-thalassemia mutations in the multi ethnic Indian population. *Blood Cells Molecules Dis* 2009; *42* : 241-6.

36. Colah R, Gorakshakar A, Phanasgaonkar S, D'Souza E, Nadkarni A, Surve R, *et al*. Epidemiology of β-thalassaemia in Western India: mapping the frequencies and mutations in sub-regions of Maharashtra and Gujarat. *Br J Haematol* 2010; *149* : 739-47.

37. Traeger J, Winichagoon P, Wood WG. Instability of β^E-messenger RNA during erythroid cell maturation in hemoglobin E homozygotes. *J Clin Invest* 1982; *69* : 1050-3.

38. Winichagoon P. Fucharoen S, Weatherall OJ, Wasi P. Concomitant inheritance of α-thalassemia in β-thalassaemia/HbE. *Am J Hematol* 1985; *20* : 217-22.

39. Nuntakarn L, Fucharoen S, Fucharoen G, Sanchaisuriya K, Jetsrisuparb A, Wiangnon S. Molecular, hematological and clinical aspects of thalassemia major and thalassaemia intermedia associated with Hb E-beta-thalassemia in Northeast Thailand. *Blood Cells Mol Dis* 2009; *42* : 32-5.

40. Pootrakul P, Vongsmasa V, La-ongpanich P, Wasi P. Serum ferritin levels in thalassemias and the effect of splenectomy. *Acta Haematol* 1981; *66* : 244-50.

41. Pootrakul P, Huebers HA, Finch CA, Pippard MJ, Cazzola M. Iron metabolism in thalassemia. *Birth Defects* 1988; *23* : 3-8.

42. Pootrakul P, Sirankapracha P, Hemsorach S, Moungsub W, Kumbunlue R, Piangitjagum A, *et al*. A correlation of erythrokinetics, ineffective erythropoiesis, and erythroid precursor apoptosis in Thai patients with thalassemia. *Blood* 2000; *96* : 2606-12.

43. Sonakul D, Pacharee P, Laohaphand T, Fucharoen S, Wasi P. Pulmonary artery obstruction in thalassemia. *Southeast Asian J Trop Med Public Health* 1980; *11* : 516-23.

44. Fucharoen S, Youngchaiyud P, Wasi P. Hypoxaemia and the effect of aspirin in thalassemia. *Southeast Asian J Trop Med Public Health* 1981; *12* : 90-3.

45. Sripichai O, Makarasara W, Munkongdee T, Kumktoek C, Nuchprayoon I, Chuansumrit A, *et al*. A scoring system for the classification of β-thalassemia/Hb E disease severity. *Am J Hematol* 2008; *83* : 482-4.

46. Nuinoon M, Makarasara W, Mushiroda T, Setianingsih I, Wahidiyat PA, Sripichai O, *et al*. A genome-wide association identified the common genetic variants influence disease severity in β-thalassemia/hemoglobin E. *Hum Genet* 2010; *127* : 303-14.

47. Gilman JG, Huisman THJ. DNA sequence variation associated with elevated fetal Gg globin production. *Blood* 1985; *66* : 783-7.

48. Labie D, Pagnier J, Lapoumeroulie C, Rouabhi F, Dunda-Belkhodja O, Chardin P, *et al*. Common haplotype dependency of high Gα-globin gene expression and high Hb F levels in β-thalassemia and sickle cell anemia patients. *Proc Natl Acad Sci USA* 1985; *82* : 2111-4.

49. Thein SL, Menzel S, Peng X, Best S, Jiang J Close J, *et al*. Intergenic variants of HBS1L-MYB are responsible for a major quantitative trait locus on chromosome 6q23 influencing fetal hemoglobin levels in adults. *Proc Natl Acad Sci USA* 2007; *104* : 11346-51.

50. Lettre G, Sankaran VG, Bezerra MA, Araujo AS, Uda M, Sanna S, *et al*. DNA polymorphisms at the BCL11A, HBS1L-MYB, and β-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. *Proc Natl Acad Sci USA* 2008; *105* : 11869-74.

51. Menzel S, Garner C, Gut I, Masuda F, Yamaguchi M, Heath S, *et al*. A QTL influencing F cell production maps to a gene encoding a zinc-finger protein on chromosome 2p15. *Nat Genet* 2007; *39* : 1197-9.

52. Sampietro M, Thein SL, Contreras M, Pazmany L. Variation of HbF and F-cell number with the G-gamma Xmn I (C-T) polymorphism in normal individuals. *Blood* 1992; *79* : 832-3.

53. Kuliev AM. The WHO control program for hereditary anemia. *Birth Defects* 1988; *23* : 383-94.

54. Cao A, Rosatelli C. Control of β-thalassemia in Sardinia. *Birth Defects* 1988; *23* : 395-404.

55. Loukopoulos D, Kaltsoya-Tassiopoulou A, Fessas P. Thalassemia control in Greece. *Birth Defects* 1988; *23* : 405-16.

56. Angastiniotis M. Cyprus: thalassemia programme. *Lancet* 1990; *2* : 1119-20.

57. Parnsatienkul B. Thalassemia. In: *current situation and strategic plan for prevention and control of blood diseases in Thailand 1989-1990*. Bangkok: Num-Aksorn Karnpim; 1990. p. 5-43.

58. Modell B, Berdoukas V. *The clinical approach to thalassemia*. London: Grone and Stratton; 1984. p. 275-7.

59. Tongsong T, Wanapirak C, Sirivatanapa P, Sanguansermsri T, Sirichotiyakul S, Piyamongkol W, *et al*. Prenatal control of severe thalassemia: Chiang Mai strategy. *Prenat Diagn* 2000; *20* : 229-34.

60. Winichagoon P, Svasti S, Munkongdee T, Chaiya W, Boonmongkol P, Chantrakul N, *et al*. Rapid diagnosis of thalassemias and other hemoglobinopathies by capillary electrophoresis system. *Transl Res* 2008; *152* : 178-84.

61. Kulapongs P, Sanguansermsri T, Mertz G, Tawarat S. Dichlorophenolindophenol (DCIP) precipitation test: a new screening test for Hb E and H. *Pediatr Soc Thailand* 1976; *15* : 1-7.

62. Kattamis C, Efremov G, Pootrakul S. Effectiveness of one tube osmotic fragility screening in detecting β-thalassemia trait. *J Met Genet* 1981; *18* : 266-70.

63. Pintar J, Skikne BS, Cook JD. A screening test for assessing iron status. *Blood* 1982; *59* : 110-3.

64. Fucharoen G, Sanchaisuriya K, Sae-Ung N, Dangwibul S, Fucharoen S. A simplified screening strategy for thalassaemia and haemoglobin E in rural communities in south-east Asia. *Bull World Health Organ* 2004; *82* : 364-72.

65. WHO. World Health Assembly Resolution on Genomics and World Health. WHA57 13 WHO, Geneva, Switzerland. Available from: *www.who/int.gb*.

Reprint requests: Dr Suthat Fucharoen, Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University
Salaya Campus, 25/25 Phuttamonthon 4 Rd, Phuttamonthon, Nakhonpathom 73170, Thailand
e-mail: grsfc@mahidol.ac.in