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Original Article

Phenotypic diversity in beta-HbE thalassemia patients

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ABSTRACT

Background Thalassemia is a monogenic disease, yet the clinical manifestations (phenotype) are variable although they have the same genotype. The clear-cut correlation between genotype and phenotype in β-thalassaemia/HbE patients remains unexplained. There are several factors that play a role in the severity of the clinical manifestations, i.e. two alpha-gene deletion, homozygote *Xmn*1 polymorphism +/+, -+-++, ++-++ haplotype, and hemoglobin Constant Spring.

Objective To understand the clinical diversity of patients with HbE/α thalassemia and to determine whether it is possible to predict phenotypic severity from genetic factors.

Methods A descriptive study on clinical presentations and hematological data of beta-HbE thalassemia patients. DNA analysis was performed to detect β-thalassemia mutations and the ameliorating factors (alpha-globin genes deletions and *Xmn1* restriction site polymorphism at position –158 upstream of the $^{G}\gamma$-globin gene) which were already known.

Results Thirty patients with HbE/β thalassemia (4 to 29 years old) were recruited. IVS1-nt5 (G>C) severe β^{+} mutation was detected in 20 patients. Eighteen of 20 patients with positive IVS1-nt5 mutation group were heterozygous for *Xmn*1 restriction site polymorphism and none of the patients was co-inherited with two á-globin gene deletion. Almost all patients (19/20) with positive IVS1-nt5 mutation group required regular transfusions, yet the mean age at first blood transfusion was older in negative IVS1-nt5 mutation group than that of positive IVS1-nt5 mutation group (5.7 vs 4 years). Mean hemoglobin before initial transfusion was higher in negative IVS1-nt5 mutation group than that of positive IVS1-nt5 mutation group (5.88 vs 5.39 g/dl). The mean total transfusion per year was lower in the negative IVS1-nt5 mutation group than that of positive IVS1-nt5 mutation group (190.6 vs 215.1 ml/year).

Conclusions Beta-HbE thalassemia patients with identical beta thalassemia mutation (IVS1-nt5) show remarkable clinical diversity. Neither two alpha-gene deletion, nor the *Xmn*1-$^{G}\gamma$ polymorphism can explain the phenotypic variation. Other ameliorating determinants or genetic modifications responsible for the variable clinical severity remain to be explored. **[Pediatr Indones 2006;46:82-86].**

Keywords: thalassemia, phenotype, mutation

Thalassemia is a monogenic disease, however, the clinical manifestations (phenotype) are variable (ranging from a mild form of thalassemia intermedia to transfusion dependent) although identical in genotypes. Clear and detail knowledge of the relationship between genotype and phenotype in beta-HbE thalassemia patients will aid the clinician in providing optimal counseling, establishing a more accurate prenatal diagnosis, and most importantly, offering better management for beta-HbE thalassemia patients.[1,2]

The clear-cut correlation between genotype and phenotype in beta-thalassaemia/HbE patients remains unexplained. Few of the homozygote-beta or heterozygote beta-HbE thalassemia patients with severe mutation have mild clinical manifestations. There are several factors that play a role in the severity of the clinical manifestations of beta-HbE thalassemia patients i.e, two alpha-gene deletion, homozygote *Xmn*1 polymorphism +/+, -+-++, ++-++ haplotype, and hemoglobin Constant Spring. The mechanism of high level HbF in beta thalassemia patients remains

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unclear. In some cases, there are mutations at promoter area in gamma globin gene, or major deletion which involves cluster beta-globin chains.[1-4]

The aim of this study was to determine the clinical diversity of patients with HbE/α thalassemia and to determine whether it is possible to predict phenotypic severity from genetic factors.

Methods

This was a descriptive study. All beta-HbE thalassemia patients who visited the Thalassemia Center at Cipto Mangunkusumo Hospital during September-November 2004 were enrolled in this study by consecutive sampling. Clinical severity was assessed based on criteria modified from Ho *et al*.[5] Major thalassemia was defined as blood transfusion began at the age of <2 years old or >2 years old with transfusion frequency of 12 times per year. Mild thalassemia intermedia was defined as hemoglobin level of >7 g/dl that could be maintained without transfusion or transfusion frequency was less than once every 2 years. Mild thalassemia intermedia was defined if transfusion was given at the incidence of less than 6-months and started after the age of 10 years with or without mild thalassemic facies observed. Severe thalassemia intermedia was defined if transfusion requirement began at the age of 4 years or older and needed to be given at the interval between 6 weeks and 4 months, it was also defined as the transfusion given at the interval of 4 months and started before the age of 4 years with classical physical appearance of thalassemic disease. Those with transfusion requirements between the two groups were classified as moderate thalassemia intermedia.[5]

Peripheral blood examination and hemoglobin analysis were carried out using the standard laboratory techniques described in a previous report.[6] Complete blood count and red cell indices were obtained by an electronic cell counter. Hemoglobin electrophoresis was carried out on cellulose acetate gels in tris-ethylenediaminetetraacetic acid-borate buffer. HbA2 and HbE were determined by microculum (Biorad) and HbF by alkaline denaturation.

Genomic DNA was isolated from 5 ml of blood colected in ethylenediaminetetraacetic acid using the Progenome kit (Progen). Alternatively, 500 µl of blood was processed for PCR analysis after cell lysis and treatment with proteinkinase-K; 2 µl of this solution was added to the PCR reaction mix. DNA for pulsed field gel electrophoresis was prepared from fresh lymphocytes immobilised and lysed in agarose blocks.

Each DNA sample was tested by PCR amplification using allele-specific priming (ARMS) for known mutations previously described in Indonesian population. Each DNA was amplified using allele-specific primer, followed by digestion using restriction enzyme Cac81 and MnlI for IVS1-nt5 (G→C), Cd 26/HbE mutation detection, respectively. Only patients with IVS1-nt5 (G→C) mutation were further analyzed. PCR amplification of the deletion breakpoint was performed using allele specific primers to detect SEA, Fil, Tha type alpha-globin gene mutation. The *Xmn*I-Gγ polymorphism was analyzed by amplification using gene specific fragment, followed by digestion with 5-10 units of *Xmn*1 endonuclease.

Results

There were 30 beta-HbE thalassemia patients aged 4-29 years, consisted of 14 males and 16 females. Twenty patients have positive IVS1-nt5 (G→C) mutation. There was not alpha-globin gene deletion in positive IVS1-nt5 mutation group; but heterozygote *Xmn*1 polymorphism +/- was detected in 18 of 20 patients and homozygote *Xmn*1 polymorphism -/- in other 2 patients. Eighteen of 20 patients of positive IVS1-nt5 mutation group came to hospital because of pale as the chief complaint, abdominal enlargement (7/20), icteric (9/20), and fever (5/20). Nineteen of 20 patients required regular transfusions.

Of negative IVS1-nt5 mutation group, 8/10 came to hospital due to pale, 4/10 abdominal enlargement, 8/10 icteric, and 2/10 patients with fever.

Comparison of several clinical parameters on beta-HbE thalassemia patients based on the status of IVS1-nt5 mutations can be seen on **Table 1**. One of 20 patients of positive IVS1-nt5 mutations group and 5/10 patients in negative IVS1-nt5 mutations group got the first blood transfusion at the age of older than 5 years old. Total PRC transfusion=240 ml/kg/year were noted in 6/19 patients of positive IVS1-nt5 mutations group and in 2/10 patients of negative IVS1-nt5 mutations group.

TABLE 1. COMPARISON OF CLINICAL PARAMETERS IN BETA-HBE THALASSEMIA PATIENTS BASED ON THE STATUS OF IVS1-NT5 MUTATIONS

Clinical parameter	IVS1-nt5 mutations	
	Positive n=20 Mean (range)	Negative n=10 Mean (range)
Hemoglobin before first transfusion (g/dL)	5.39 (2.7-8.7)	5.88 (3.5-9.7)
HbF level (%)	17.5 (2.6-45.7)	25.08 (3.9-61.3)
HbE level (%)	33.72 (15.1-63.8)	40.56 (16.7-70.0)
The ages of first blood transfusion (year)	4 (1-11)	5.7 (1-12)
Transfusion frequency per year	11 (6-12)	12 (6-16)
Total PRC transfusion (ml/kg/year)	215.1 (88-450)	190.6 (85-300)
Ferritin (ng/mL)	5670.2 (992-24.650)	4490.3 (1.582-12.000)

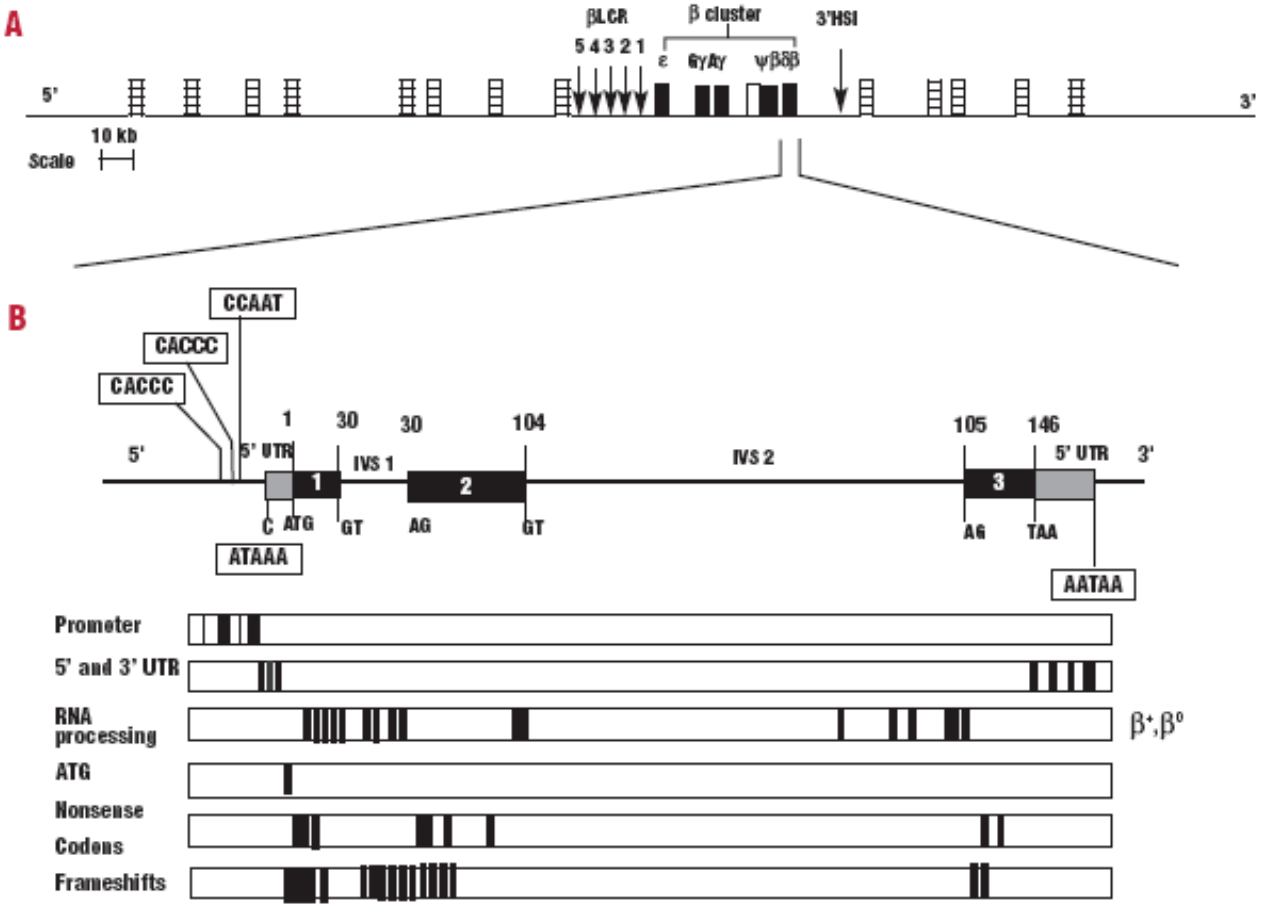

FIGURE 1. A. THE B GLOBIN GENE CLUSTER AND ITS FLANKING REGIONS ON CHROMOSOME 11P. THE E, GG, AG, D AND B GLOBIN GENES ARE INDICATED AS GRAY BOXES. THE 5' HYPERSENSITIVE SITES (1 TO 5) WHICH COMPRISE THE BLCR AND THE 3' HYPERSENSITIVE SITES (3'HS1) ARE SHOWN AS VERTICAL ARROWS. HATCHED BOXES REPRESENT THE OLFACTORY RECEPTOR GENES. B. GENERAL STRUCTURE OF THE B GLOBIN GENE WITH THE 3 EXONS (GRAY BOXES) AND THE 2 INTERVENING SEQUENCES (IVS1 AND IVS2). CONSERVED SEQUENCES (DETAILED IN TEXT) ARE INDICATED. THE DIFFERENT CLASSES OF POINT MUTATIONS CAUSING B THALASSEMIA ARE SHOWN BELOW THE B GLOBIN GENES.[9]

Discussion

In Thailand, the prevalences of beta-thalassemia and beta-HbE thalassmia are 3-9% and 13% respectively (in the Northeast of Thailand the prevalence is up to 50-70%).[3,7] Rees *et al*[8] reported that beta-HbE thalassemia is frequent in Thailand, Indonesia, India, Bangladesh, and Srilangka.

There are several mutations of beta-chains, some of them can be seen on **Figure 1**.[9] In Thailand, the most common mutation is 41/42 frame-shift (50.9%), meanwhile IVS-1 nt5 mutation is only found in 5.2% of patients. IVS1-nt5 (G→C) was the most common mutation found in China, India, Malaysia, and Indonesia with the prevalence 48.3%, 22.5%, 48.8% and 35.3% respectively.[7,9,10] We found IVS1-nt5 (G→C) mutation in 20 of 30 patients (66.7%).

We found beta-HbE thalassemia patients with positive mutation had more severe clinical manifestations. In our study, the positive IVS1-nt5 mutation group had lower mean hemoglobin level before transfusion, younger age of first blood transfusion, lower HbF and HbE level, and higher total transfusions per year than that of negative mutation group. As mentioned above, there were several factors that play a role in the severity of the clinical manifestation of beta-HbE thalassemia patients due to two alpha-gene deletion, homozygote *Xmn*1 polymorphism +/+ (it elevates production of gamma globin chain which appear to have higher HbF level), -+-++, ++-++ haplotype, and hemoglobin Constant Spring.[1-4] Factors which can elevate production of gamma globin chains are 1). intrinsic propensity, 2) 5' beta promoter deletion, 3). δ γ thalassemia deletion, 4) hemoglobin Lepore.[1,11,12]

Winichagoon *et al*[4] reported that homozygote *Xmn*1-^{G}γ polymorphism +/+ has strong correlation with high HbF level and high hemoglobin production in 2/46 mild beta-HbE thalassemia patients. Homozygote *Xmn*1-^{G}γ polymorphism +/+ has higher hemoglobin level, HbF level, and G γ/A ã ratio than that of heterozygote *Xmn*1-^{G}γ polymorphism +/- or homozygote *Xmn*1-^{G}γ polymorphism -/-.[3] Of 90 beta-HbE thalassemia patients, 36 patients had mild clinical manifestations. Fifteen of 36 patients had homozygote *Xmn*1-^{G}γ polymorphism +/+ or mild beta thalassemia genotype or alpha thalassemia or hemoglobin Constants Spring.[3] Setianingsih found that all of 66 beta-HbE thalassemia patients had heterozygote *Xmn*1-^{G}γ polymorphism +/-.[6]

Body temperature or infection also play a role on HbE stability that the higher body temperature, the more unstable HbE. Not only the unstable HbE can make red blood cells lysis but also oxidative stress.[8]

We did not find two alpha-gene deletion in positive IVS1-nt5 mutation group; only heterozygote *Xmn*1-^{G}γ polymorphism +/- in 18 of 20 patients and homozygote *Xmn*1-^{G}γ polymorphism -/- in 2 of 20 patients. We found that neither heterozygote *Xmn*1-^{G}ã polymorphism +/- nor homozygote -/- could ameliorate natural history of beta-HbE thalassemia. We did not check the haplotype due to limitation of facility. The clinical manifestations in our study showed remarkable diversity, ranging from mild form to transfusion dependent (**Table 1**). Total transfusion of more 240 ml/kg per year was noted in 6/19 patients of positive IVS1-nt5 mutation group and in 2/10 patients of negative IVS1-nt5 mutation group.

Due to small sample size, we cannot see the role of homozygote *Xmn*1-^{G}γ polymorphism +/+ in ameliorating the clinical manifestations of beta-HbE thalassemia patients. Further study should be done to detect the type of haplotype. The role of infection and nutrition on the phenotype of beta-HbE thalassemia patients should also be explored.

In conclusion, beta-HbE thalassemia patients with identical β thalassemia mutation (IVS1-nt5) showed remarkable clinical diversity. Neither two alpha-gene deletion, nor the *Xmn*1-^{G}γ polymorphism explained the phenotypic variation. Other ameliorating determinants or genetic modifications responsible for the variable clinical severity remain to be explored.

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