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LETTER TO THE EDITOR

Frequency of hemoglobin E/β-thalassemia compound heterozygotes with low hemoglobin F phenotype among cases with a diagnosis of hemoglobin E homozygote, determined by high-performance liquid chromatography, in prenatal control program for β-thalassemia

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Dear Editor,

Hemoglobin (Hb) E (HBB:c.79G>A)/β-thalassemia disease is the most common thalassemia syndrome in Southeast Asian countries with a high prevalence of Hb E. Even though patients could present with a wide spectrum of clinical severity, the disease accounts for half of the severe β-thalassemia patients worldwide [1]. Among individuals with a carrier state of β-thalassemia or Hb E and homozygous Hb E who could be at-risk couples for Hb E/β-thalassemia, individuals with Hb E/β-thalassemia themselves, especially with mild severity, may still be able to have their own children and also could be at-risk couples. The problem could occur with few Hb E/β-thalassemia compound heterozygotes who have a very low Hb F phenotype and could be misdiagnosed with Hb E homozygote by Hb analysis [2–4]. Consequently, if their spouses had inherited Hb E allele, they could be at-risk couples for Hb E/β-thalassemia. To date, there are no data regarding the frequency of Hb E/β-thalassemia cases with a low Hb F phenotype in real-life prenatal control situations.

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Our study was conducted prospectively in prenatal control program for β-thalassemia in the lower north of Thailand, between February 2014 and May 2017. All couples with a phenotypic diagnosis of homozygous Hb E by high-performance liquid chromatography (HPLC: VARIANT™) in one person, and heterozygous or homozygous Hb E in the other, were recruited. Phenotypic diagnosis of Hb E homozygote comprised of a major fraction of Hb E with Hb F proportion less than 10%, without Hb A. DNA methods to confirm their genotype of Hb E homozygote and to detect other β-thalassemia mutations [5, 6], together with α^0-thalassemia (Southeast Asian and Thai deletions) and α^+-thalassemia (3.7- and 4.2-kb deletions) determinants [7], were performed in all samples with Hb E homozygote phenotype. The study was approved by the institutional ethical committee. Of the 6023 couples determined by HPLC, there were 792 subjects with a phenotype of Hb E homozygote identified. Among these, 464 couples met our requirement, including 25 with double diagnoses of Hb E homozygote. The mean (± SD) Hb E and Hb F proportions in 489 Hb E homozygotes were 77.87 ± 5.27 and 3.54 ± 1.82%, respectively. After performing genotypic diagnosis, five (1.0%) Hb E/β-thalassemia subjects were identified (Fig. 1). All five samples had co-inherited either α^0-thalassemia or α^+-thalassemia allele. Furthermore, all five cases had a spouse who had inherited Hb E, meaning they were at-risk couples for Hb E/β-thalassemia nearly misdiagnosed (Table 1).

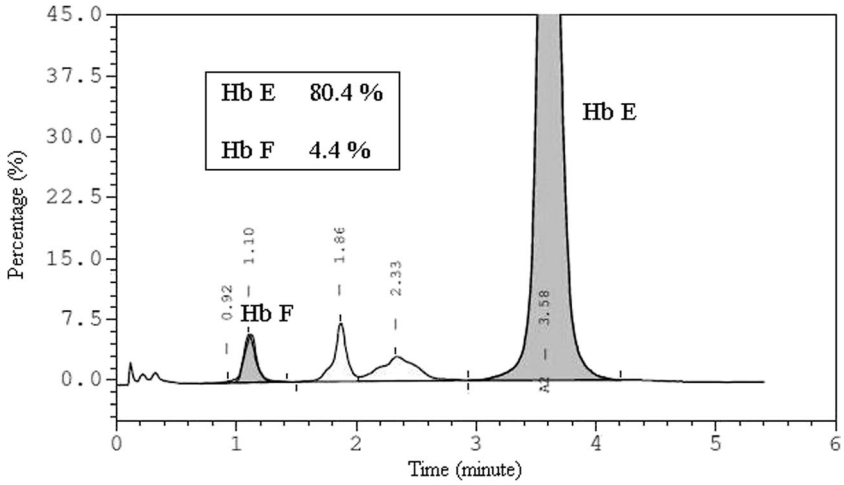

Fig. 1 A representative chromatogram of a hemoglobin E/β-thalassemia subject with low hemoglobin F phenotype. *Hb* hemoglobin

With this false negative limitation of HPLC (1.0%), all Hb E homozygote individuals who have a spouse with any Hb E phenotypes must have their diagnoses confirmed prior to thalassemia counseling. Capillary electrophoresis method can discriminate Hb E/β-thalassemia from Hb E homozygote in a certain number of cases by using a level of Hb A_2 which co-separates with Hb E by HPLC. However, there are also some overlapping values of Hb A_2 in these two conditions, depending on several factors [3, 4].

In process of globin assembly, αβ dimers form in preference to other dimers due to the equivalence of positive and negative charges. In situations in which reduced α-globin chain production occurs, the effect of the charge becomes exaggerated [8]. By observation, Hb E homozygote and Hb E/β-thalassemia cases with concomitant α-thalassemia always have a reduction in Hb F values [3, 9]. One study could demonstrate Hb F level reduction in Hb E homozygote (8.3 ± 6.3 versus 3.1 ± 3.9%) and Hb E/β^0-thalassemia (34.8 ± 15.1 versus 9.9 ± 4.2%) cases who co-inherited with two α-globin gene defects. In addition, the same study could even observe the elevation of the Hb E fraction in Hb E/β^0-thalassemia cases (58.2 ± 13.7 versus 72.0 ± 10.4%) [3]. Concordant with our findings, these results may imply that the reduced α-globin chains (from concomitant α-thalassemia) preferentially bind to the mutated β- (β^E) in a much higher proportion than γ-globin chains. However, due to the relatively small number of Hb E/β-thalassemia subjects diagnosed with low Hb F phenotype (five cases) identified in our study, there could still be other possible factors besides α-thalassemia determinant in the lowering cause of Hb F in individuals with Hb E/β-thalassemia which were outside the scope of our study.

Table 1 Hematological and molecular characteristics of the five hemoglobin E/β-thalassemia subjects with low hemoglobin F phenotype

Subject	1	2	3	4	5
Hb (g/dL)	11.4	8.5	10.8	–	–
Hct (%)	38.4	30.0	35.0	–	–
MCV (fL)	52.0	59.0	49.7	50.5	46.8
MCH (pg)	15.5	16.9	15.9	14.5	–
Hb A_2/E (%)	83.0	74.6	74.4	73.8	80.4
Hb F (%)	3.0	3.7	3.9	9.9	4.4
β-Globin mutation[a]	Codon95 (+A)	Codon41/42 (−TTCT)	Codon41/42 (−TTCT)	Codon17 (A>T)	Codon71/72 (+A)
α-Thalassemia genotype	$--^{SEA}/\alpha\alpha$	$--^{SEA}/\alpha\alpha$	$--^{SEA}/\alpha\alpha$	$--^{SEA}/\alpha\alpha$	$-^{3.7}\alpha/\alpha\alpha$

Hb, hemoglobin; *Hct*, hematocrit; *MCV*, mean corpuscular volume; *MCH*, mean corpuscular hemoglobin; $-^{SEA}$, Southeast Asian deletion; $-^{3.7}$, 3.7-kb deletion

[a] *In trans* to the hemoglobin E mutation

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Compliance with ethical standards The study was approved by the institutional ethical committee.

Conflict of interest The authors declare that they have no conflict of interest.

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