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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).

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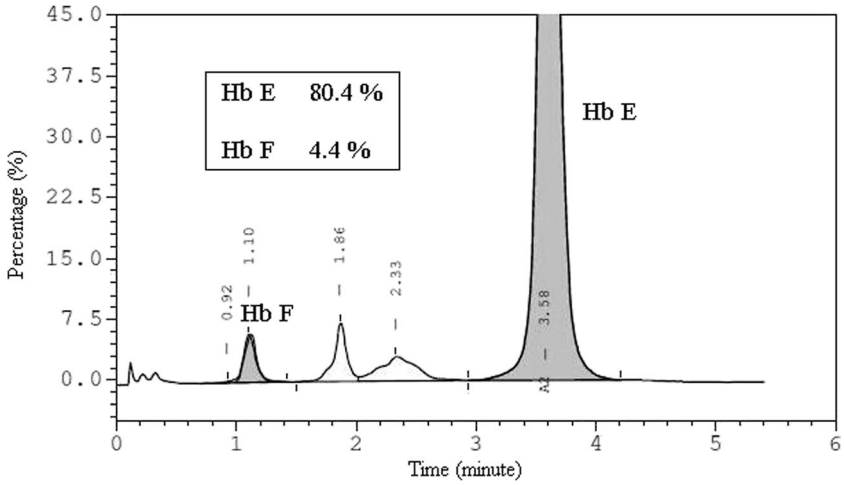

Fig. 1 A representative chromatogram of a hemoglobin E/β-thalassemia subject with low hemoglobin F phenotype. *Hb* hemoglobin

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.

References

1. Olivieri NF, Pakbaz Z, Vichinsky E (2010) HbE/β-thalassemia: basis of marked clinical diversity. Hematol Oncol Clin North Am 24: 1055–1070
2. Yamsri S, Singha K, Prajantasen T, Taweenan W, Fucharoen G, Sanchaisuriya K, Fucharoen S (2015) A large cohort of β(+)-thalassemia in Thailand: molecular, hematological and diagnostic considerations. Blood Cells Mol Dis 54:164–169
3. Sae-ung N, Srivorakun H, Fucharoen G, Yamsri S, Sanchaisuriya K, Fucharoen S (2012) Phenotypic expression of hemoglobins A_2, E and F in various hemoglobin E related disorders. Blood Cells Mol Dis 48:11–16
4. Prasing W, Pornprasert S (2014) Measurement of $HbA_2$by capillary electrophoresis for diagnosing β-thalassemia/HbE disease in patients with low HbF. Lab Med 45:226–230
5. Saetung R, Ongchai S, Charoenkwan P, Sanguansermsri T (2013) Genotyping of beta thalassemia trait by high-resolution DNA melting analysis. Southeast Asian J Trop Med Public Health 44:1055–1064
6. Sirichotiyakul S, Saetung R, Sanguansermsri T (2003) Analysis of beta-thalassemia mutations in northern Thailand using an automated fluorescence DNA sequencing technique. Hemoglobin 27:89–95
7. Sanguansermsri T, Phumyu N, Chomchuen S, Steger HF (1999) Screening for alpha-thalassemia-1 heterozygotes in expecting couples by the combination of a simple erythrocyte osmotic fragility test and a PCR-based method. Community Genet 2:26–29
8. Steinberg MH, Adams JG (1991) Hemoglobin A_2: origin, evolution, and aftermath. Blood 78:2165–2177
9. Pakdee N, Yamsri S, Fucharoen G, Sanchaisuriya K, Pissard S, Fucharoen S (2014) Variability of hemoglobin F expression in hemoglobin EE disease: hematological and molecular analysis. Blood Cells Mol Dis 53:11–15