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Phenotypic expression of hemoglobins A_2, E and F in various hemoglobin E related disorders

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ABSTRACT

Study on the phenotypic expression of hemoglobin (Hb) A_2 and Hb E in Hb E disorders has been difficult due to the co-separation of Hb A_2 and Hb E in most Hb analysis assays. Because these two Hbs are separated on capillary electrophoresis, we studied phenotypic expression of Hbs A_2, E and F in various Hb E disorders using this system. This was done on 362 subjects with several Hb E disorders including heterozygous Hb E, homozygous Hb E, β-thalassemia/Hb E, δβ-thalassemia/Hb E, and Hb Lepore/Hb E and those of these disorders with several forms of α-thalassemia. Normal controls showed Hb A_2 of $2.7 \pm 0.3\%$. Heterozygous Hb E and homozygous Hb E had elevated Hb A_2 i.e. $3.8 \pm 0.3\%$ and $4.8 \pm 0.5\%$, respectively. Further elevations were observed for β^0-thalassemia/Hb E ($6.1 \pm 1.9\%$) and β^+-thalassemia/Hb E ($7.1 \pm 1.2\%$). Interestingly, no elevation of Hb A_2 was found in the δβ-thalassemia/Hb E, and Hb Lepore/Hb E ($2.3 \pm 0.3\%$) but higher Hb F levels were noted which could be useful diagnostic markers. The levels of Hb E were variable. Co-inheritance of these Hb E disorders with α-thalassemia were associated with lower outputs of Hb E and Hb F but the levels of Hb A_2 were not altered. Different phenotypic expression of Hb A_2, Hb E and Hb F could help in differential diagnosis of these Hb E related disorders commonly encountered in the regions where access to molecular techniques is limited.

Introduction

Hemoglobin E (Hb E) is the most common hemoglobinopathy among Southeast Asian population. Significant numbers of Hb E are also found in India, Bangladesh and Sri Lanka. The overall prevalence of Hb E in Thailand is about 20–30%, with the highest frequency of about 50% observed in northeastern Thai population [1,2]. The β^E chain is synthesized at a reduced rate as compared to β^A chain because the β^E mutation (β26; GAG-AAG) creates an abnormal splicing within the exon 1 of the β-globin gene [3]. It is symptomless in heterozygote but homozygote can have a mild hemolytic anemia and microcytosis. Interactions of Hb E with α- and β-thalassemias can lead to several thalassemia syndromes with varying clinical and hematological phenotypes. Interactions could result in change in hematological features and misdiagnosis during routine investigation [4–6]. Study on the phenotypic expression of Hb A_2 and Hb E in Hb E related disorders has been difficult due to the co-separation of Hb A_2 with Hb E in most Hb analysis assays. However, it has been shown that a capillary electrophoresis system could separate Hb A_2 from Hb E and many other abnormal Hbs [7–10], the characteristic useful for screening, identification and diagnosis of Hb E syndromes. In this study, we investigated the hematological features and phenotypic expression of Hbs A_2, E and F in several Hb E related disorders using this capillary electrophoresis system and demonstrated that accurate measurements of these Hbs could help in providing differential diagnosis of these common genetic disorders in the region.

Materials and methods

Subjects and hematological analysis

The study protocol was approved by the Institutional Review Board of Khon Kaen University, Khon Kaen, Thailand (HE 501109). A total of 323 blood specimens of Thai individuals with several Hb E related disorders were selectively recruited from our ongoing thalassemia screening program at Centre of Research and Development of Medical Diagnostic Laboratories, Khon Kaen University, Thailand. Hematological parameters were determined using an automated blood cell counter (Coulter T series; Beckman-Coulter Co., CA, USA). Hb analysis was performed using automated capillary electrophoresis (Capillarys 2; Sebia, Lisses, France) [7–10] in which each separated Hb is detected spectrophotometrically at 415 nm. Normal and abnormal Hb variants are observed from the cathode to the anode in the

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following sequence: Hbs Constant Spring or Paksé, A_2, E, F, A, Bart's and H. Hbs A_2, E and F were respectively identified in zones 3, 4 and 7 as shown in Fig. 1.

DNA analysis

Identification of six common α-thalassemia alleles including α^0-thalassemia (SEA and THAI deletions), α^+-thalassemia (3.7 kb and 4.2 kb deletions) and Hb Constant Spring or Hb Paksé mutations were routinely performed in our laboratory using gap-PCR and allele specific PCR (ASPCR) as described elsewhere [11–13]. ASPCR assays were routinely carried out for identification of Hb E and β-thalassemia mutations in our laboratory [14–16]. Identification of δβ-thalassemia and hereditary persistence of fetal Hb (HPFH) in Thailand was performed using a multiplex PCR method as described previously [17].

Statistical analysis

Statistical comparison of hematological parameters was performed with the Kruskal–Wallis and the Mann–Whitney *U*-test using the Minitab statistical software (Minitab Inc., State College, Pa., USA). A *P*-value of less than 0.05 was considered statistically significant.

Results

Fig. 1 demonstrated representatively the capillary electrophoregrams of Hb E heterozygote, Hb E homozygote, Hb E-β^0-thalassemia and Hb E–δβ-thalassemia. As shown in the figure, Hbs A_2, E and F were clearly separated on this assay, allowing us to directly compare the levels of them in various Hb E disorders. Since α-globin genotypes can alter the levels of these Hbs, hematological phenotypes of the subjects were therefore analyzed according to both β^E and α-globin genotypes as shown in Table 1. To simplify this analysis, α^0-thalassemia caused by a double α-globin gene deletion and a compound heterozygous α^+-thalassemia caused by a single gene deletion was classified as the 2 α-globin gene defect. Heterozygous α^+-thalassemia, Hb Constant Spring or Hb Paksé was grouped into a 1 α-globin gene defect. Accordingly, interaction of α^0-thalassemia and α^+-thalassemia causing the Hb H disease was defined as the 3 α-globin gene defect. The level of Hb A_2 in normal adult determined using capillary electrophoresis in our laboratory is 2.7 ± 0.3% (data not shown).

Among 143 heterozygous Hb E, only 78 had normal α-globin genotype (αα/αα), the remaining had co-inherited α-thalassemia. The levels of Hb A_2 and Hb E in these 78 pure heterozygous Hb E were found to be 3.8 ± 0.3% and 25.6 ± 0.9%, respectively. Hb F was not elevated (<2%). The elevated Hb A_2 levels were still observed in those of heterozygous Hb E with 1 and 2 α-globin gene defects (3.7 ± 0.3% and 3.9 ± 0.4%) but not in those with 3 α-globin gene defect (2.9 ± 0.7%). Interestingly, significant reductions in Hb E were respectively observed in those of heterozygous Hb E with these 1, 2 and 3 α-globin gene defects (25.6 ± 0.9% *v.s.* 23.7 ± 1.8%, 16.6 ± 1.6% and 11.9 ± 2.0%). Hb F was again less than 2% in all cases.

Further elevation of Hb A_2 (ranging from 4.6 to 7.0%) was observed for 72 homozygous Hb E either with or without α-thalassemia. Hb F was elevated (~7–8%) although a significant reduction was noted for homozygous Hb E with 2 α-globin gene defects (8.3 ±

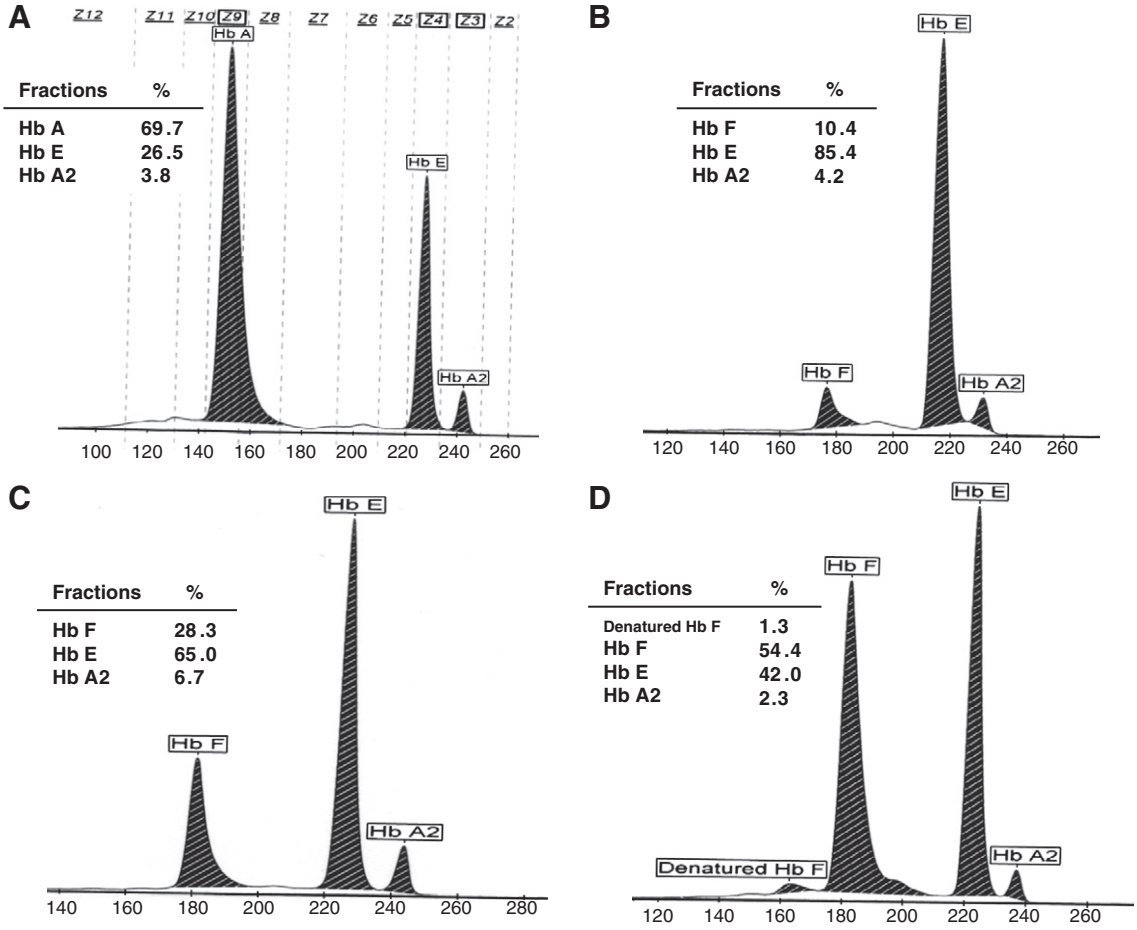

Fig. 1. Representative capillary electrophoregrams of adult subjects with (A) heterozygous Hb E; (B) homozygous Hb E; (C) Hb E–β^0-thalassemia; and (D) Hb E–$\delta\beta^0$-thalassemia. Hb A, Hb A_2, Hb E and Hb F are indicated.