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Globin Chain Analysis by HPLC and Restriction Fragments Aanysis of Polymerase Chain Reaction (PCR) Amplified DNA for Detection of HbE Mutation

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Hemoglobin E(HbE) is the most common hemoglobinpathy in Southeast Asia. Patients who have compound heterozygous HbE trait and β°-thalassemia trait are often dependent on transfusions like β-thalassemia patient. Because HbE can't be differential from hemoglobin A_2 by microcolumn chromatographic method, which is the standard method used in clinical laboratories for detection of β-thalassemia carrier. Globin chain analysis by high performance liquid chromatography (HPLC) and restriction fragments analysis for detecion of HbE mutation from dried blood spot samples collected on filter paper, which is used in our matermal screening program, were developed.

Dried blood spot samples of one homozygous HbE patient and normal cases were collected. Reverse phasse C4 column was used on HPLC to analyze globin chains. After eluted from the dried blood spots, 20 μg hemoglobin was injected to the HPLC. On the chromatoghram, the peaks of α, β, Gγ, and Arglobin chains showed good symmetry and resolution. The precision of the retention time and concentration were 3% and 3.7%, respectively. The chromatogram of the confirmed HbE patients showed a unique peak of β^E globin chain separated from β. Because the (G→A) mutation of HbE results a recognition sequence of Mnl I, the endonuclease was used for the restriction fragments analysis of DNA amplified froom the dried blood spots by PCR. HbE mutation was characterized by a 232 base pairs fragment while the normal allele produced 172 and 60 base pairs fragments. This method has been applied to five cases with HbA_2% higher than 15% detected by our β-thalassemia carrier screening program and four of them were found to be HbE carriers. This study successfully showed a way to identify HbE mutation from dried blood spot specimens and may be adapted by clinical laboratory to be used with whole blood samples.

140