Detection of Hemoglobin New York [β 113 (G15) Val→Glu, GTG>GAG] in a Thai Woman by Capillary Electrophoresis

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Abstract Hemoglobin (Hb) New York [β 113 (G15) Val→Glu, GTG>GAG] is a very rare β -chain variant found in Thailand. This variant is often missed by routine laboratory testing because Hb New York and Hb A have the identical retention time on high performance liquid chromatography. We reported here for the first time that the detection of Hb New York in a Thai woman by using capillary electrophoresis (CE). A peak of Hb New York located ahead of Hb A at the electrophoretic zone 11 with a level of 42.8 %. The DNA sequencing revealed the GTG>GAG mutation at codon 113 for Hb New York on one allele of β -globin gene. Therefore, the CE has a high efficiency to prevent the misinterpretation of hemoglobin analysis in patients who are heterozygote of this variant.

Keywords Detection · Capillary electrophoresis · Hb New York · High performance liquid chromatography · Thai woman

Images in Hematology

A 25-year-old Thai woman was seen by an obstetrician at Lanna hospital Chiang Mai, Thailand when she was in her 16th week of gestation. Her blood sample was collected

with EDTA as anticoagulant and hematological parameters were measured by the ADVIA 2120i hematology analyzer (Siemens Healthcare Diagnostic, Deerfield, IL, USA). Values observed were: WBC 5.3×10^9 cells/L, RBC 4.7×10^{12} cells/L, Hb 114 g/L, PCV 0.36 L/L, MCV 77.0 fL, MCH 24.5 pg, MCHC 320 g/L, and platelet 284×10^9 /L. The hemoglobin analyzed by capillary electrophoresis (CE, CAPILLARYSTM 2, Sebia, Norcross, Georgia, USA) showed Hb A 54.5 % and Hb A₂ 2.7 %. In addition, an abnormal Hb peak with a level of 42.8 % was observed at the electrophoretic zone 11 (Fig. 1a). Her hemoglobin typing was also further analyzed by high performance liquid chromatography (HPLC, VARIANT II, β -thalassemia Short Program, Bio-Rad Laboratories, Hercules, California, USA). The Hb F, Hb A, and Hb A₂ were presented at levels of 0.4, 87.2, and 3.0, respectively while the abnormal Hb peak was not observed on HPLC chromatogram (Fig. 1b). The molecular analysis for detection of the α -thalassemia-1 South-East Asian (SEA) and Thai type deletion was performed by using the real-time PCR with SYBR Green1 and high resolution melting (HRM) analysis [1]. Furthermore, the α -thalassemia-2 ($-\alpha^{3.7}$ and $-\alpha^{4.2}$ kb deletions) was also analyzed by using the multiplex Gap-PCR [2]. The results were negative for both α -thalassemia-1 and -2 deletions. In order to identify the abnormal Hb, the PCR amplification of the β -globin gene was accomplished and then the direct DNA sequencing of the amplified product was performed on an ABI PRISMTM 3130 XL analyzer (Applied Biosystems, Foster City, CA, USA). The results showed the molecular defect causing abnormal hemoglobin resulting in a T to A transversion at the second position of codon 113 (GTG>GAG) of β -globin gene (Fig. 2) that leads to a substitution of glutamic acid for valine as described previously for Hb New York [β 113 (G15) Val→Glu, GTG>GAG] [3].

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Fig. 1 The HPLC chromatogram (a) and CE electrophoregram (b) of the proband

Fig. 2 Representative DNA sequence derived from the proband

Hb New York is a silent variant with normal hematological indices. This variant can be missed on HPLC because it is unable to be separated from Hb A. It can be identified and quantified by CE method and its peak located ahead of Hb A at the electrophoretic zone 11. The heterozygote of Hb New York is clinically and hematologically normal. However, the compound heterozygote of Hb New York and Hb S presented complication associated to sickling [4]. Even though Hb S is very rare in Thailand, the β -thalassemia and other β -globin variants such as Hb E, Hb Hope, and Hb Tak are highly frequent [5]. The compound heterozygote of Hb New York and β -thalassemia or these β -globin variants may lead a clinical picture as thalassemia intermedia with mild anemia. Therefore, the ability for detection of Hb New York will be used to assist in management and counseling for patients.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

Informed consent This study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand. The informed consent was obtained from all individual participants included in the study.

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