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Diagnostic Challenges of Hemoglobin (Hb) Hekinan with deletional α^0-thalassemia and β^0-thalassemia During Prenatal Screening for Fetal Hb E/β-Thalassemia Risk

Keywords: Hb Hekinan; Deletional α^0-thalassemia; β^0-thalassemia; Prenatal thalassemia screening.

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To the editor.

Thalassemia is a leading genetic problem in Southeast Asian countries including Thailand. During routine prenatal thalassemia screening, structural variants of hemoglobin (Hb), with or without a thalassemia phenotype, can be consistently found. A large cohort study conducted by a thalassemic center in the northeast of Thailand identified 2.4% of Hb variants (excluding Hb E and Hb Constant Spring) in all diagnostic samples, mainly consisting of 16 and 13 unique mutations in the β- and α-globin chains, respectively.[1] Despite a relatively small number of Hb variants, interaction of a variant with more prevalent thalassemia alleles results in a complex thalassemia syndrome, which may pose difficulties in routine diagnosis, especially in determining at-risk couples during pregnancy. This is a case report of a pregnant woman who has a complex interplay between a rare α-globin mutation and two common α- and β-thalassemia alleles, which is difficult to identify during prenatal thalassemia screening. The purpose of this case report is to demonstrate the advantage of employing a combination of high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) for screening of the abnormal Hb.

In a prenatal diagnosis program for thalassemia, a blood sample from a 28-year-old pregnant woman was sent to Naresuan University Hospital for Hb analysis at 12 weeks gestation after antenatal care. Her complete blood count showed: Hb 12 g/dL; hematocrit 37%; mean corpuscular volume (MCV) 66 fL; mean corpuscular hemoglobin 22 pg; mean corpuscular hemoglobin concentration 33 g/dL; and red cell distribution width 15%. Hb analysis using HPLC (VARIANT, BioRad, Hercules, USA) (**Figure 1A**) demonstrated a normal Hb A_2 fraction (2%) and an abnormal peak that was not completely separated from Hb A, at 31.7% at a retention time of 2.29 minutes. However, Hb analysis using CE (Minicap Flex, Serbia, Lisses, France) (**Figure 1B**) revealed a normal A_2A pattern with 5.6% of Hb A_2 fraction, and no abnormal Hb was detected. Based on the results of her chromatogram and electropherogram, it could be summarized that she was diagnosed with β-thalassemia heterozygote and unknown Hb variant. Routine DNA analysis in prenatal thalassemia screening program using polymerase chain reaction (PCR) method was performed to identify common mutations of β-thalassemia and α^0-thalassemia [Southeast Asian (SEA) and THAI deletion].[2] Her results showed as expected that she was a carrier of β^0-thalassemia [codon 41/42 frameshift mutation (-CTTT); HBB:c.125_128 delCTTT] and unprecedented α^0-thalassemia [SEA deletion; NG_000006.1: g.26264_45 564del19301]. Since Hb A was presented in a β^0-thalassemia heterozygote subject (represented wild-type β-globin gene *in trans* to the mutant β-allele), the unknown Hb variant should belong to α-globin chain mutation. Therefore, α-globin DNA analysis was performed using a multiplex allele-specific PCR assay described elsewhere[3] and revealed Hb Hekinan [α27(B8)Glu→Asp, GAG>GAT(α1); HBA1:c.84G>T] (**Figure 2**). Direct DNA sequencing of α-1 globin gene demonstrated the G>T transition at codon 27 (**Figure 3**). As her spouse was Hb E heterozygote, the fetus was at risk for Hb E/β^0 thalassemia. Prenatal genetic testing was offered to the couple, but the results were unavailable.

Hb Hekinan is a non-pathological α-globin chain variant (normal function and stability).[4] The point mutation in the α-1 globin gene creates an amino acid substitution from glutamic to aspartic acid which retains the charge and size, but only shortens the side chain which may not affect Hb molecule.[4,5] This is a rare Hb variant reported in Japanese, Chinese and Taiwanese populations.[6,7] Hb Hekinan has also been reported in Thailand combining with several common hemoglobinopathies in South-east Asia, including

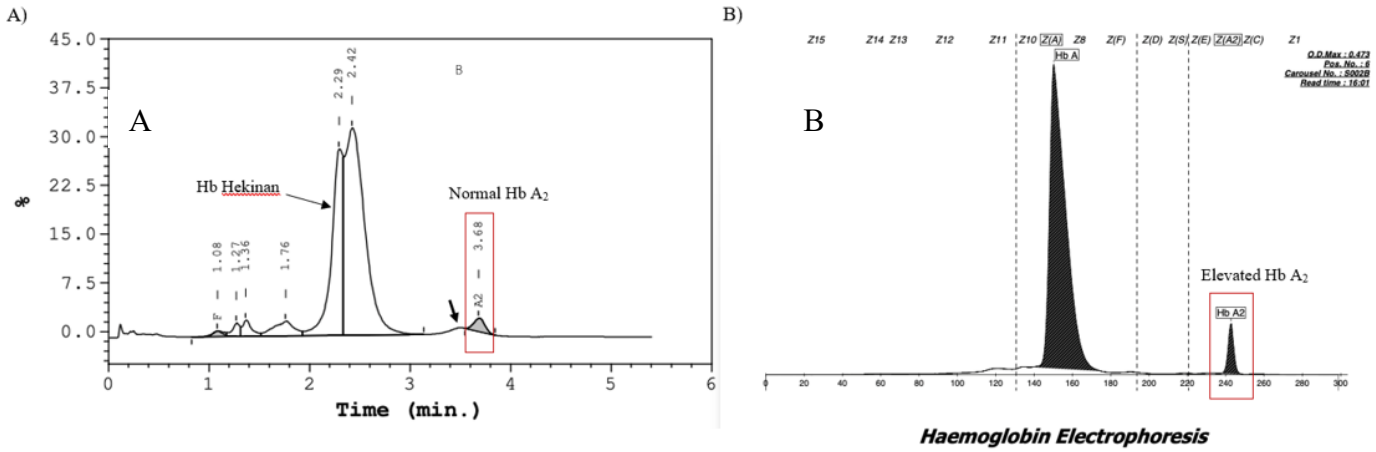

Figure 1. A) Hemoglobin (Hb) analysis by automated high-performance liquid chromatography shows an abnormal peak (thin arrow) beside Hb A that is not completely separated from Hb A in the amount of 31.7% and a small projection (thick arrow) preceding Hb A_2 suspected of variant Hb A_2. **B**) Capillary electrophoresis demonstrates a normal electropherogram with an elevated Hb A_2 level. **Abbreviations:** Hb, hemoglobin; min, minutes

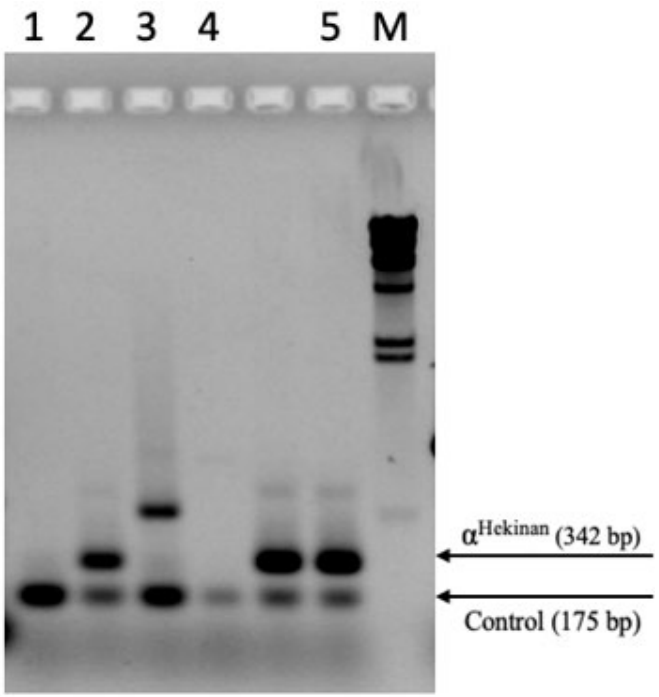

Figure 2. Identification of the hemoglobin (Hb) Hekinan mutation by multiplex allele-specific polymerase chain reaction. Lane 1: negative control, lane 2: Hb Hekinan carrier, lane 3: Hb Nakhon Ratchasima carrier, lane 4: Hb Phnom Penh carrier, lane 5: this patient. **Abbreviation:** bp, base pair.

deletional α^0-thalassemia (SEA deletion), deletional α^+-thalassemia (-$\alpha^{3.7}$ deletion) and Hb E.[5,8-10] However, the coexistence of Hb Hekinan with deletional α^0-thalassemia (SEA deletion) and β^0-thalassemia has not been previously reported.

We reported an undescribed complex condition until now in which Hb Hekinan interacted with deletional α^0-thalassemia (SEA deletion) and β^0-thalassemia alleles in a healthy pregnant woman. She had a normal Hb level but low MCV value. Her chromatogram from HPLC showed a normal Hb A_2 fraction with Hb Hekinan that could not be completely separated from Hb A and her electropherogram from CE demonstrated co-migration of Hb Hekinan and Hb A (**Figure 1**).[8] This intricate interaction did not result in the formation of the Hb H phenotype as previously reported.[11] The explanation in this case must be from a normal $\alpha^{Hekinan}$-globin chain production combined with a β-globin chain reduction from β-thalassemia allele.[9]

Currently, mass screening of β-thalassemia carriers by determining their Hb A_2 fraction is a routine practice in detecting at-risk couples during pregnancy. Severe iron deficiency, and α- as well as δ-thalassemia coinheritance are the main factors that reduce the production of α- and δ-globin chains, which cause lower levels of Hb A_2, possibly resulting in an undetectable β-thalassemia carrier.[11-15] Since iron studies are not part of our routine antenatal care for patients with normal Hb level, mild iron deficiency which is unlikely to affect Hb A_2 level was not excluded in this subject. Discrepancy of Hb A_2 results can be explained by the different separation principles of the two analytic platforms. In this present study, HPLC analysis revealed normal level of Hb A_2. However, due to the results of high Hb A_2 fraction demonstrated by CE, the cause of Hb A_2 reduction in HPLC must be due to the separation of Hb A_2 variant from normal Hb A_2, as indicated by a minor uninterpreted Hb projection (suspected of $\alpha_2^{Hekinan}/\delta_2$ tetramer) just before the normal Hb A_2 fraction in chromatogram; the high Hb A_2 level in CE actually was due to normal Hb A_2 and variant Hb A_2 co-migrated.[16] Similar to a case study describing the complex interaction of Hb Hekinan with deletional α^{+0}-thalassemia and Hb E, an apparent Hb A_2/E variant peak with the same retention time, preceding the normal A_2/E fraction, was identified in HPLC.[5] Co-inheritance with Hb E results in a prominent Hb A_2/E variant peak because Hb E co-elutes with Hb A_2 on HPLC, making the abnormal Hb fraction (variant Hb A_2 and variant Hb E combined) more readily recognizable.[5,9,17] Compared to the present case, the absence of variant Hb E leaves the small amount of variant Hb A_2 more challenging to detect during prenatal β-thalassemia carrier screening.[7]

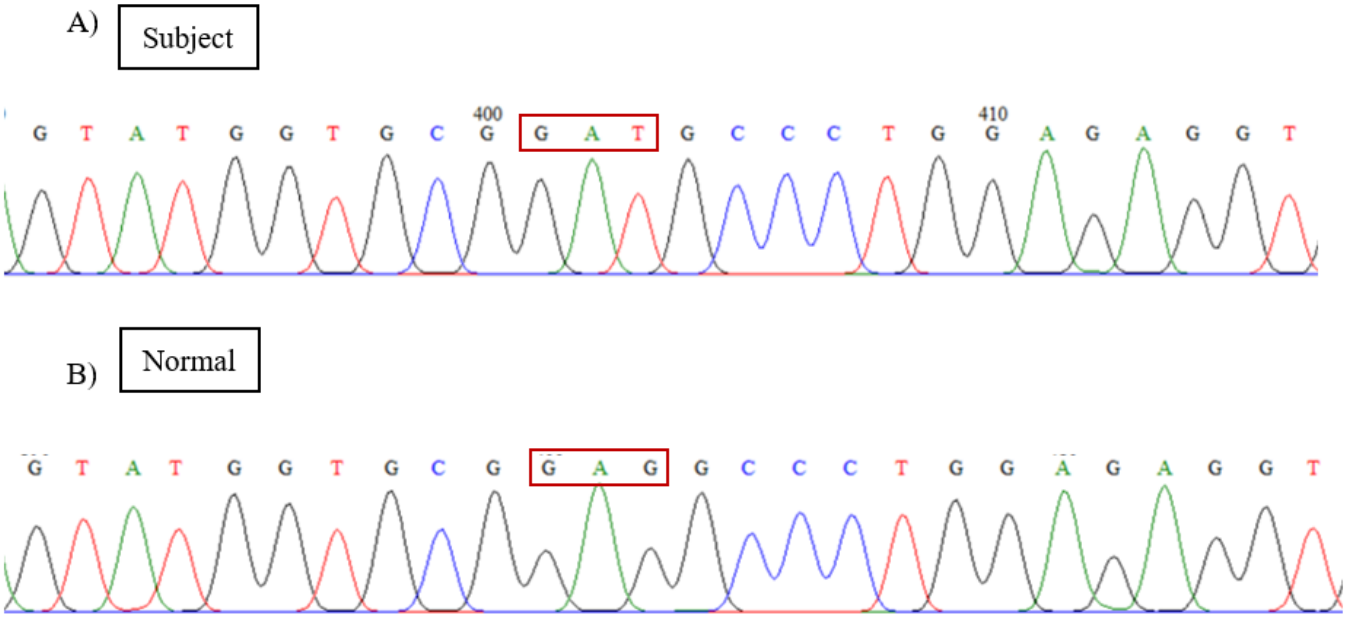

Figure 3. Direct DNA sequencing of α-1 globin gene in this subject demonstrates the GAG>GAT mutation at codon 27 causing the hemoglobin Hekinan (**A**) comparing with normal (**B**).

Due to her husband's diagnosis of Hb E heterozygote, the couple was finally confirmed to be at risk for Hb E/β^0 thalassemia. Therefore, co-inheritance of Hb Hekinan and α^0-thalassemia alleles may have further implications for population screening of β-thalassemia carriers. The diagnostic resolution of Hb Hekinan and other Hb variants in prenatal thalassemia screening might be more accurate by using a combination of Hb analyzers or using isoelectric focusing (IEF), which provides clearer separation of Hb Hekinan from Hb A.[10]

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Ethics approval and consent to participate. This patient provided written broad informed consent for blood sampling and for the collection and reporting of research data, in accordance with the Declaration of Helsinki and with approval from the Institutional Review Board of Naresuan University (NU-IRB #2577).

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Competing interests: The authors declare no competing interest.

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