Case Report

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Coinheritance of hemoglobin D-Punjab and β⁰-thalassemia 3.4 kb deletion in a Thai girl

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Abstract:

Hemoglobin (Hb) D-Punjab [β121(GH4) Glu→Gln; HBB: C.364G>C] and β⁰-thalassemia 3.4 kb deletion are very rare in the Thai population. For the first time, the coinheritance of HbD-Punjab with β⁰-thalassemia 3.4 kb deletion was reported in a 7-year-old Thai girl. She had mild anemia (Hb 115.0 g/L and mean corpuscular hemoglobin 18.1 pg) with red blood cell microcytosis (mean corpuscular volume 52.5 fL). By capillary electrophoresis (CE), HbD-Punjab was found at a migration position of 180 s with the value of 81.9% while the level of HbA_2 was 7.3%. Based on the elevated HbA_2, the molecular analysis for detection of β⁰-thalassemia mutations was performed. The 490 bp amplified fragments from β⁰-thalassemia 3.4 kb deletion was observed. Thus, the coinheritance of HbD-Punjab with β⁰-thalassemia can be found in the Thai population. The HbA_2 measured on CE is a reliable parameter for differentiating the homozygote of HbD-Punjab and compound heterozygote of HbD-Punjab and β⁰-thalassemia.

Keywords:

Capillary electrophoresis, coinheritance, hemoglobin A_2, hemoglobin D-Punjab, β⁰-thalassemia 3.4 kb deletion

Introduction

Hemoglobin (Hb) D-Punjab [β121(GH4) Glu→Gln; HBB: C.364G>C] is a Hb variant carrying an amino acid substitution at position 121 of the β-globin chain. It was also known as HbD-Los Angeles, HbD-North Carolina, HbD-Portugal, HbD-Chicago, and Hb Oak Ride.[1] HbD-Punjab is quite prevalent in Pakistan, Northwest India, China, Middle East countries, and also in many other parts of the world with the overall frequency of 0.2%–3.0%.[2-4] Both the heterozygote and homozygote for HbD-Punjab are clinically silent conditions. However, the coinheritance of HbD-Punjab with HbS [β6(A3) Glu→Val; HBB: C.20A>T] results in mild clinical symptoms to severe conditions with sickle cell disease.[5] Moreover, the coinheritance of HbD-Punjab with HbE [β26(B8) Glu→Lys; HBB: C.79G>A] or with β⁺-thalassemia had been reported in Thai patients. The typical thalassemic indices with hypochromic microcytosis (mean corpuscular hemoglobin [MCH] 18.6–25.5 pg and mean corpuscular volume [MCV] 57.0–77.2 fL) were observed in these patients.[1] The coinheritance of HbD-Punjab with β⁰-thalassemia is not common. There has been no report on the overall prevalence of coinheritance of HbD-Punjab with β⁰-thalassemia. However, the prevalence of compound heterozygosity for HbD-Punjab and β-thalassemia reported at the Hematology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran from 2006 to 2011 was 0.6%,[6] and a few cases of compound heterozygosity for HbD-Punjab and β⁰-thalassemia were reported in India, Saudi Arabia, and England.[2,4,7] These cases had mild to severe anemia with hepatosplenomegaly, Hb

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values between 80 and 120 g/L, and typical thalassemic indices and morphological change of red cells. HbF values are slightly elevated (1%–7%) while HbA_2 values are varied from 2.9% to 6.4%.[2,7] The normal value of HbA_2 in a sample with coinheritance of HbD-Punjab and β^0-thalassemia could result in the misdiagnosis of β-thalassemia carrier and inappropriate genetic counseling, as in the case reported by Belhoul *et al*.[8] In this study, we reported for the first time of the coinheritance of HbD-Punjab with β^0-thalassemia in a Thai girl.

Case Report

A 7-year-old Thai girl was seen by a pediatrician at Hatyai Hospital, Songkhla, Thailand for annual health check-up. Her blood sample was collected with ethylenediaminetetraacetic acid as anticoagulant and the complete blood count was analyzed using an automated blood counter (Sysmex KX-21, Sysmex Corporation, Kobe, Japan). Values observed were white blood cell 5.89 × 10^9 cells/L, red blood cell (RBC) 6.34 × 10^{12} cells/L, Hb 115.0 g/L, packed cell volume 0.33 L/L, MCV 52.5 fL, MCH 18.1 pg, MCH concentration 345 g/L, red cell distribution width 18.4%, and platelet 420 × 10^9/L. Her blood sample was also sent to the Associated Medical Sciences Clinical Service Center, Chiang Mai University, Chiang Mai, Thailand, for the thalassemia diagnosis. In the thalassemia laboratory, the Hb analysis was performed using high-performance liquid chromatography (HPLC,

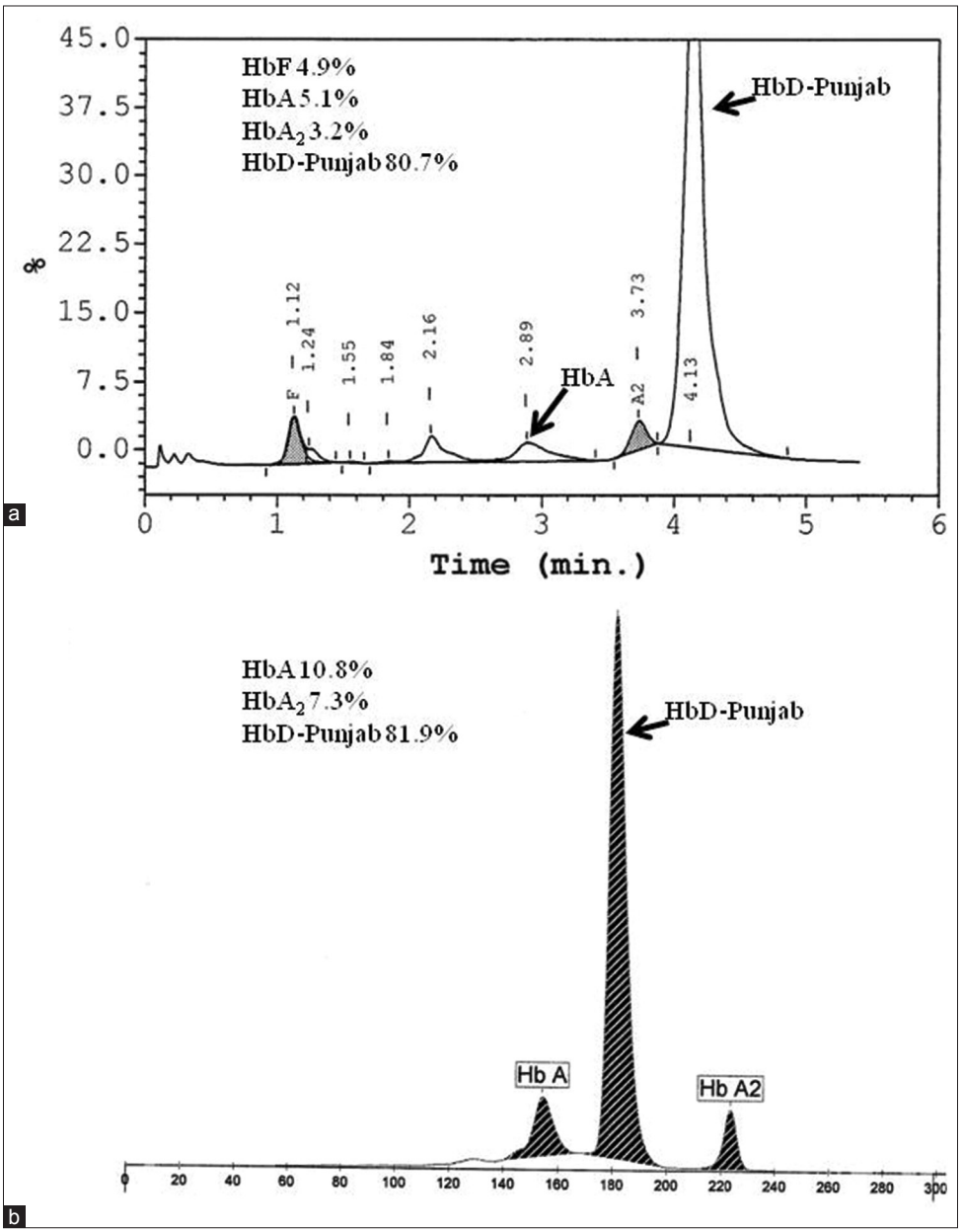

Figure 1: The high-performance liquid chromatography chromatogram (a) and capillary electrophoresis electrophoregram (b) of the patient

VARIANT II, β-thalassemia Short Program, Bio-Rad Laboratories, Hercules, California, USA). The abnormal Hb peak with a value of 80.7% was observed in D-window at the retention time of 4.13 min while the levels of HbA, HbA_2, and HbF were 5.1%, 3.2%, and 4.9%, respectively [Figure 1a]. The Hb analysis was also performed by capillary electrophoresis (CE, Capillarys™ 2 Flex Piercing, Sebia, Norcross, Georgia, USA). On the CE electrophoregram, the abnormal Hb with a value of 81.9% was presented at a migration position of 180 s while the levels of HbA and HbA_2 were 10.8% and 7.3%, respectively [Figure 1b].

The real-time polymerase chain reaction (PCR) with SYBR Green1 and high resolution melting analysis for detection of the α-thalassemia-1 Southeast Asian and Thai type deletions[9] was also performed in this sample and the negative analysis result was observed. The multiplex allele-specific-PCR was performed for molecular diagnosis of Hb Q-Thailand (HBA1: C.223G>C) and three β-globin variants, including Hb Tak (HBB: C.441_442insAC), HbS (HBB: C.20A>T), and HbD-Punjab because these Hb variants are eluted at the same retention time on HPLC chromatogram and have a similar migration position on CE electrophoregram.[10] The 329 bp amplified fragment from the HbD-Punjab allele was observed [Figure 2a]. The elevated HbA_2 (7.3%) was found on the CE electrophoregram. Therefore, the coinheritance of HbD-Punjab with β⁰-thalassemia was doubted. The β⁰-thalassemia codons 17 (A>T), 41/42 (−TCTT), 71/72 (+A), and IVSI nt1 (G>T) mutations were analyzed by the multiplex amplification refractory mutation system-PCR, whereas the β⁰-thalassemia 3.4 kb deletion was detected by the Gap-PCR, according to protocols described previously.[11] The 490 bp amplified fragment of β⁰-thalassemia 3.4 kb deletion was observed [Figure 2b]. Thus, the patient was finally diagnosed as coinheritance of HbD-Punjab with β⁰-thalassemia 3.4 kb deletion.

Discussion

HbD-Punjab and β⁰-thalassemia 3.4 kb deletion are not common in Thailand. The prevalence of β⁰-thalassemia 3.4 kb deletion varies from 0.3% to 4.3%.[9] Thus, this is the first case of coinheritance of HbD-Punjab with β⁰-thalassemia 3.4 kb deletion reported in a Thai subject. She had mild anemia with RBC microcytosis, but she did not have hepatosplenomegaly. Consistency with the previous study showed that cases with coinherited HbD-Punjab and β⁰-thalassemia had only mild to moderate anemia.[2] In this study, the HbA_2 level measured by HPLC was 3.2% which was in the reference ranges (<3.5%). On HPLC chromatogram, the normal (3.3%) and elevated (6.4%) HbA_2 levels had been reported in two siblings who

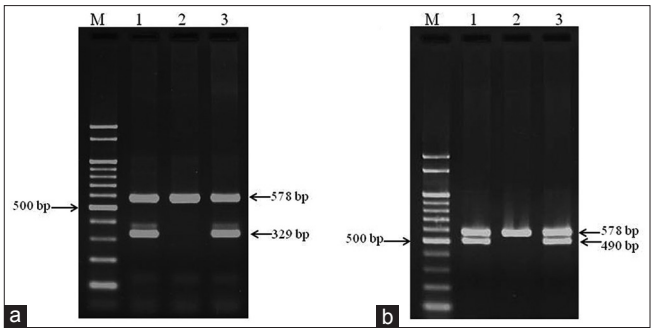

Figure 2: Representative agarose gel electrophoresis for: (a) The allele-specific-polymerase chain reaction for detection of the hemoglobin D-Punjab. The produce fragments of 329 and 578 bp specific for hemoglobin D-Punjab and internal control fragment, respectively, are indicated. Lane 1: Hemoglobin D-Punjab carrier; Lane 2: normal control; Lane 3: the subject. M represents the GeneRuler 100 bp DNA ladder. (b) The Gap-polymerase chain reaction for detection of β⁰-thalassemia 3.4 kb deletion. The produce fragments of 490 and 578 specific for β⁰-thalassemia 3.4 kb deletion and internal control fragment, respectively, are indicated. Lane 1: β⁰-thalassemia 3.4 kb deletion positive control, Lane 2: normal control; Lane 3: the subject. M represents the GeneRuler 100 bp DNA ladder

coinherited HbD-Punjab with the same β⁰-thalassemia mutation (codon 30, AGG>ACG).[7] These results suggested that HbA_2 level measured on HPLC which is used as a diagnostic marker for β-thalassemia trait is not a reliable parameter when differentiating homozygotes of HbD-Punjab and compound heterozygotes of HbD-Punjab and β⁰-thalassemia. However, the elevated HbA_2 (7.3%) was observed on the CE electrophoregram. On HPLC, HbD-Punjab elutes close to HbA_2 generally lead to an underestimated HbA_2 level. On CE, common Hb variants including HbS, HbC, HbD-Punjab, and HbE migrate separately from HbA_2 and they do not interfere in the HbA_2 quantification.[12] Thus, CE is very efficient in separating and quantifying HbA_2 and it is a reliable method for differentiating homozygotes of HbD-Punjab and compound heterozygotes of HbD-Punjab and β⁰-thalassemia.

Conclusion

The coinheritance of HbD-Punjab with β⁰-thalassemia can be found in the Thai population. Therefore, a better understanding of HPLC chromatogram and CE electrophoregram patterns and clinical features of this combination is useful for genetic counseling, prevention, and control programs for thalassemia and hemoglobinopathy.

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Conflicts of interest

There are no conflicts of interest.

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