Case Report

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 HbA2 was 7.3%. Based on the elevated HbA2, 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 HbA2 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 A2, 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 HbA2 values are varied from 2.9% to 6.4%. The normal value of HbA in a sample with coinheritance of HbD-Punjab and β0-thalassemia could result in the misdiagnosis of β-thalassemia carrier and inappropriate genetic counseling, as in the case reported by Belhoul et al. In this study, we reported for the first time of the coinheritance of HbD-Punjab with β0-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 × 10⁹ cells/L, red blood cell (RBC) 6.34 × 10¹² 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 × 10⁹/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,
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, HbA₂, and HBF were 5.1%, 3.2%, and 4.9%, respectively [Figure 1a]. The Hb analysis was also performed by capillary electrophoresis (CE, Capillaries™ 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 HbA₂ 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 HbA₂ (7.3%) was found on the HPLC chromatogram. 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 IVS1 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.[9] In this study, the HbA₂ 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%) HbA₂ levels had been reported in two siblings who coinherited HbD-Punjab with the same β⁰-thalassemia mutation (codon 30, AGG>ACG).[7] These results suggested that HbA₂ 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 HbA₂ (7.3%) was observed on the CE electrophoregram. On HPLC, HbD-Punjab elutes close to HbA₁ generally lead to an underestimated HbA₂ level. On CE, common Hb variants including HbS, HbC, HbD-Punjab, and HbE migrate separately from HbA₂ and they do not interfere in the HbA₂ quantification.[12] Thus, CE is very efficient in separating and quantifying HbA₂ 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.

References
1. Fucharoen S, Changtrakun Y, Surapot S, Fucharoen G, Sanchaisuriya K. Molecular characterization of Hb D-Punjab [beta121(GH4) Glu→Gln] in Thailand. Hemoglobin 2002;26:261-9.
2. Das S, Mashon RS. Coinheritance of Hb D-Punjab and β-thalassemia: Diagnosis and implications in prenatal diagnosis. Hemoglobin 2015;39:138-40.
3. Torres Lde S, Okumura JV, Silva DG, Bonini-Domingos CR. Hemoglobin D-Punjab: Origin, distribution and laboratory diagnosis. Rev Bras Hematol Hemoter 2015;37:120-6.
4. Worthington S, Lehmann H. The first observation of Hb D Punjab beta zero thalassaemia in an English family with 22 cases of unsuspected beta zero thalassaemia minor among its members. J Med Genet 1985;22:377-81.
5. Torres LS, Okumura JV, Belini-Júnior É, Oliveira RG, Nascimento PP, Silva DG, et al. Phenotypic diversity of sickle cell disease in patients with a double heterozygosity for Hb S and Hb D-Punjab. Hemoglobin 2016;40:356-8.
6. Haghpanah S, Ramzi M, Zakerinia M, Nourani Khojasteh H, Haghsenas M, Rezaei N, et al. Epidemiology of hemoglobinopathies and thalassemias in individuals referred to the haematology research centre, Shiraz University of Medical Sciences, Shiraz, Iran from 2006 to 2011. Hemoglobin 2014;38:287-8.
7. Owaidah TM, Al-Saleh MM, Al-Hellani AM. Hemoglobin D/ beta-thalassemia and beta-thalassemia major in a Saudi family. Saudi Med J 2005;26:674-7.
8. Belhoul KM, Bakir ML, Abdulrahman M. Misdiagnosis of Hb D-Punjab/ß-thalassemia is a potential pitfall in hemoglobinopathy screening programs: A case report. Hemoglobin 2013;37:119-23.
9. Pornprasert S, Wiengkum T, Srithep S, Chainoi I, Singboottra P, Wongwiwatthanakul S. Detection of α-thalassemia-1 Southeast Asian and Thai type deletions and β-thalassemia 3.5-kb deletion by single-tube multiplex real-time PCR with SYBR Green1 and high-resolution melting analysis. Korean J Lab Med 2011;31:138-42.
10. Sanchaisuriya K, Chunpanich S, Fucharoen G, Fucharoen S. Multiplex allele-specific PCR assay for differential diagnosis of Hb S, Hb D-Punjab and Hb Tak. Clin Chim Acta 2004;343:129-34.
11. Old J, Traeger-Synodinos J, Galanello R, Petrou M, Angastiniotis M. Laboratory Methods. Prevention of Thalassemias and Other Haemoglobin Disorders. 1st ed., Vol. 2. Nicosia: Team Up Creations; 2005.
12. Keren DF, Hedstrom D, Gilbranson R, Ou CN, Bak R. Comparison of Sebia capillaries capillary electrophoresis with the Primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies. Am J Clin Pathol 2008;130:824-31.