BRIEF REPORT

Iron deficiency anemia interfering the diagnosis of compound heterozygosity for Hb constant spring and Hb Paksé: The first case report

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Background: Diagnosis of thalassemia or hemoglobinopathy concomitant with iron deficiency anemia (IDA) is challenging.

Method: We report a case of 43-year-old female whose diagnosis of compound heterozygosity for hemoglobin Constant Spring (HbCS) and Hb Paksé became apparent after the treatment of IDA.

Results: Prior to treatment, Hb analysis using isoelectric focusing (IEF) showed HbA 95.6%, HbA₂ 2.7%, and HbCS 1.7% compatible with heterozygous HbCS. After 4 months of oral iron therapy resulting in an improved Hb level, her HbCS level was substantially increased to 8.7% on IEF suggesting homozygous HbCS. Subsequent DNA analysis using multiplex amplification refractory mutation system analysis revealed compound heterozygosity for HbCS and Hb Paksé.

Conclusion: This case demonstrated that IDA can significantly reduce HbCS/Hb Paksé levels and probably mask the diagnosis of homozygous HbCS, homozygous Hb Paksé or the compound heterozygosity for both hemoglobinopathies by hemoglobin analysis. The test should be repeated after resolution of IDA, or molecular testing should be performed to confirm the diagnosis.

KEYWORDS

Hb Constant Spring, Hb Paksé, hemoglobin analysis, hemoglobinopathy, iron deficiency anemia

Iron deficiency anemia (IDA) and thalassemia syndromes are two major causes of hypochromic microcytic anemia.¹ Their clinical symptoms and hematological parameters are overlapped. Therefore, the accurate diagnosis of these disorders is sometimes complicated, especially when they coexist. We report a challenging diagnosis of compound heterozygosity for Hb Constant Spring (HbCS) and Hb Paksé in a patient with iron deficiency anemia.

A 43-year-old Thai female, originating from the northeastern part of Thailand, was referred to hematology clinic to evaluate the causes of severe microcytic anemia at King Chulalongkorn Memorial Hospital. She reported progressive shortness of breath during physical activities and hypermenorrhea over the past 6 months. She had a family history of mild anemia of indeterminate cause in all of the family members. She denied any other bleeding, changes in bowel habits or dark-colored urine. She did not take any medications. She had no prior history of surgery or blood transfusion.

On physical examination, the patient was markedly pale without jaundice. She had atrophic tongue. Her spleen was palpable at 4 cm below the left costal margin. Laboratory tests showed a red cell count at $3.12 \times 10^6/\mu\text{L}$, total hemoglobin (Hb) at 3.4 g/dL, mean corpuscular volume (MCV) at 43.9 fL, red blood cell distribution width (RDW) at 23%, mean corpuscular hemoglobin (MCH) at 10.9 pg, and mean corpuscular hemoglobin concentration (MCHC) at 24.8 g/dL. The white blood cell and platelet counts were within the normal range (Table 1). Peripheral blood smear showed markedly hypochromic microcytic anemia with moderate anisopoikilocytosis. A provisional diagnosis of iron deficiency anemia with underlying thalassemia disease was made. Oral ferrous sulfate and folic acid supplement were prescribed. The results of iron study panel and hemoglobin analysis before treatment were as following: serum ferritin level 2.2 ng/L, serum iron level 4 $\mu\text{g}/\text{dL}$, TIBC 394 $\mu\text{g}/\text{dL}$, and transferrin saturation of 1%. Hemoglobin analysis was performed using isoelectric focusing (IEF) method

TABLE 1 Laboratory parameters

	At diagnosis	4 weeks after treatment	4 months after treatment	3 years after treatment
Hematologic parameters				
Red cell count ($\times 10^6/\mu\text{L}$)	3.12	4.27	3.94	4.86
Hb (g/dL)	3.4	8.7	9.5	11.3
MCV (fL)	43.9	76.8	83.5	81
RDW (%)	23.1	26.5	19.9	18.2
MCH (pg)	10.9	20.4	24.1	23.2
MCHC (g/dL)	24.8	26.5	28.9	28.7
White cell count ($\times 10^3/\mu\text{L}$)	6.06	9.90	8.60	12.05
Platelet count ($\times 10^3/\mu\text{L}$)	372	242	207	197
Hemoglobin Analysis (IEF)				
HbA (%)	95.6		88.6	
HbA ₂ (%)	2.7		2.7	
HbCS (%)	1.7		8.7	
Iron study				
Ferritin (ng/mL)	2		74.4	

and high-performance liquid chromatography (HPLC), Variant II β -thalassemia short program (Bio-Rad Laboratories, Hercules, CA, USA) to detect and determine the quantity of each Hb type. On IEF, each band of Hb variants was quantified using densitometric scanning technique, IsoScan Imaging System for Hemoglobin Testing (PerkinElmer Life and Analytical Sciences, Turku, Finland). On IEF, hemoglobin typing showed HbA 95.6% HbA₂ 2.7% and HbCS 1.7%, while HPLC showed a small peak at HbC window suggesting heterozygous HbCS. The laboratory results were interpreted as HbCS trait with iron deficiency anemia. However, splenomegaly contradicted the diagnosis of HbCS trait.

After 4 weeks of treatment, the patient's Hb level was increased to 8.7 g/dL. Her symptoms were significantly improved. Oral ferrous sulfate was continued at a replacement dose for a total of 4 months until her Hb level reached 9.5 g/dL, MCV 83.5 fL,

RDW 19.9%, and reticulocyte count 11.58%. Despite the resolution of iron deficiency anemia, the patient remained mildly anemic. The second hemoglobin analysis was repeated due to the clinical suspicion for HbHCS or homozygous CS. The Hb analysis on IEF showed HbA 88.6%, HbA₂ 2.7%, and HbCS 8.7%, while a tall sharp HbCS peak was detected on HPLC suggesting homozygous HbCS (Figure 1).

The α -globin gene analysis was performed as previously described.² The single-tube multiplex amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) analysis to differentiate HbCS ($\alpha 2$ codon 142 TAA $\rightarrow$ CAA) and Hb Paksé ($\alpha 2$ codon 142 TAA $\rightarrow$ TAT) identified both Hb variants. Further DNA analysis was performed and showed no common deletions by multiplex gap PCR method for detection of the majority of deletional α -thalassemia

FIGURE 1 HPLC chromatograms. The patient's HPLC chromatograms obtained at the diagnosis of iron deficiency anemia (HPLC Bio-Rad Variant, Left) and after 4 months of oral iron therapy (HPLC Bio-Rad Variant II, Right). The graph showed small peak at HbC window (arrow) suggesting heterozygous HbCS. However, a tall sharp HbCS peak (dotted arrow) detected after resolution of IDA suggested homozygous HbCS. HPLC, High-performance liquid chromatography; HbCS, Hemoglobin Constant Spring; IDA, iron deficiency anemia.

determinants. Therefore, the final diagnosis was compound heterozygosity for HbCS and Hb Paksé coexisting with IDA.

The patient was referred to Department of Gynecology to evaluate the cause of hypermenorrhea. She was found to have a 10-cm intramural myoma uteri. She denied surgery but treatment with depomedroxyprogesterone acetate (DMPA) injection monthly resulted in resolution of hypermenorrhea. Eventually, her Hb reached 11.3 g/dL, and oral iron supplement could be discontinued.

HbCS is the most common nondeletional α -thalassemia variant worldwide, while Hb Paksé is a rare α -globin variant found mainly in Southeast Asia. Both are the results of different point mutations at the stop codon on the $\alpha 2$ -globin gene (Codon 142 TAA→CAA in HbCS and TAA→TAT in Hb Paksé), replacing the termination codon with amino acid glutamine and tyrosine, respectively.³ The mRNA products of these mutations are very unstable leading to markedly reduced α -globin chain production. They also translate to elongated α -globin chains which precipitate on red blood cell membrane causing ineffective erythropoiesis and apoptosis, and have impaired binding to α -hemoglobin stabilizing protein.^{4,5} Clinically, both variants have indistinguishable phenotypes. Individuals with heterozygous mutation are asymptomatic and hematologically normal, while the homozygotes or combined heterozygous with deletional mutations show more severe phenotypes including mild to moderate hemolytic anemia, jaundice, and splenomegaly.^{2,3,6-8}

Differing by only one single amino acid substitution, HbCS and Hb Paksé cannot be distinguished by Hb electrophoresis or chromatography. Molecular screening in selected populations in Thailand found HbCS to be approximately 10 times more prevalent than Hb paksé (5%-5.8% vs 0.5%-1.5%).^{5,9} One study found Hb Paksé and its combinations, including compound HbCS/Hb Paksé, in 4.4% of patients who were previously diagnosed as HbCS.¹⁰ This present report underlines the importance of DNA analysis in patients who have been diagnosed as HbCS from electrophoresis or HPLC. Although these two Hb variants are similar in aspects of clinical characteristics as well as prognosis and implications to management, it is important to correctly identify the genetic diagnosis in order to provide accurate epidemiological data and genetic counseling. To date, there have been few reported cases of compound heterozygous HbCS/Hb Paksé in patients from Cambodia and northern part of Thailand.^{10,11} This may be, in part, due to the underperformed molecular diagnosis required to identify these variants. This patient's normal MCV after treatment of iron deficiency anemia also differed from the mild hypochromic microcytic characters in previously reported patients.

IDA is the most common cause of hypochromic microcytic anemia worldwide. Iron deficiency was shown to interfere with the diagnosis of β -thalassemia trait by reducing the level of HbA₂ to a normal level.^{12,13} In addition, amounts of HbE are substantially reduced when iron deficiency coexists.¹⁴ In α -thalassemia, patients who had IDA were found to have more pronounced microcytosis than patients who did not.¹⁵ However, there have been no previous reports on how iron status affects the analysis of HbCS/Hb Paksé on Hb analysis. In this patient, IDA did not affect the HbA₂

level but lowered the level of HbCS/Hb Paksé detected by IEF and HPLC. This finding could suggest the role of iron in the synthesis of HbCS/Hb Paksé, which can be more prominent than in HbA₂ as in this patient. When interpreting Hb analysis results, iron status should always be evaluated. If IDA is present, Hb analysis should be carefully interpreted. Likewise, the persistence of anemia after treatment of IDA warrants a search for underlying hemoglobinopathies. As in this case, the presence of mild hemolytic anemia and splenomegaly triggered the notion that the patient might have more severe genotypes such as homozygous HbCS or HbHCS rather than HbCS trait. Subsequent genetic analysis provided the accurate final diagnosis.

Given the different clinical characteristics and the important implications in preconception genetic counseling, it is prudent to repeat hemoglobin analysis after iron deficiency anemia subsided, or perform molecular testing to confirm the diagnosis if available.

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