Case Report/Series

Hematologic profile of hemoglobin constant spring and its co-inheritance with hemoglobin C: a case report

Sumaiyah Adzahar[1], Sharifah Sakinah Syed Abdul Rahman[1], Mohammad Hudzaifah Nordin[2], Nabilah Rameli[3]

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Authors' affiliations:
[1]Department of Pathology and Medical Laboratory, Faculty of Medicine, Universiti Sultan Zainal Abidin, Kuala Terengganu, Malaysia, [2]Faculty of Medicine, Universiti Sultan Zainal Abidin, Kuala Terengganu, Malaysia, [3]Pathology Unit, Hospital Dungun, Kuala Terengganu, Malaysia

Corresponding author:
Sumaiyah Adzahar
Department of Pathology and Medical Laboratory, Faculty of Medicine, Universiti Sultan Zainal Abidin, Jalan Sultan Mahmud, 20400 Kuala Terengganu, Terengganu, Malaysia
Tel/Fax: +60-96275546/+60-6275771
E-mail: srikandimaya11@gmail.com

ABSTRACT
Hemoglobin constant spring (Hb CS) is an alpha-globin variant characterized by an elongated alpha chain due to a mutation that leads to unstable hemoglobin (Hb) levels. Hemoglobin C (Hb C) is a beta-globin variant associated with mild hemolytic anemia. The co-inheritance of Hb CS and Hb C is uncommon and presents distinct hematologic features. We report the case of a 7-year-old Malaysian male who developed acute polyarthritis due to streptococcal infection. Routine blood tests revealed microcytic hypochromic anemia, suggestive of a thalassemia trait. Hb analysis via high-performance liquid chromatography and capillary electrophoresis detected low hemoglobin A levels with an additional peak in the Hb C zone. DNA analysis confirmed compound heterozygosity for Hb CS and Hb C. Family screening revealed that the mother is an Hb C carrier, and the father is an Hb CS carrier. This case report examined the hematological profile of a patient with this genetic combination.

KEYWORDS alpha-thalassemia, beta-globins, capillary, chromatography, electrophoresis

Thalassemia and hemoglobinopathies are the most common inherited blood disorders and represent major public health issues worldwide, including Southeast Asia.[1] Alpha-thalassemia is classified into deletional and non-deletional types. Deletional alpha-thalassemia leads to either α^0- or α^+-thalassemia, depending on the size and characteristics of the deletion. Non-deletional alpha-thalassemia occurs due to mutations in α2 or α1 genes.

Hemoglobin constant spring (Hb CS) is one of the most common non-deletional variants in Southeast Asia, affecting 1–8% of the region.[1,2] It is caused by a point mutation in the termination codon of the α2-globin gene (TAA>CAA), synthesizing unstable and elongated alpha-globin chains.[3] Hemoglobin C (Hb C) is another hemoglobin (Hb) variant with a mutation in the beta-globin gene. This case involves co-inheritance of Hb CS (alpha-globin mutation) and Hb C (beta-globin mutation), an uncommonly reported genetic profile with unique clinical and laboratory findings.

CASE REPORT

A 7-year-old Malaysian male with no prior medical illness presented with acute polyarthritis due to streptococcal infection and was treated with penicillin. His complete blood count showed Hb 15.2 g/dl, red blood cell (RBC) 6.67 × 10^{12}/l, mean corpuscular

volume (MCV) 64.6 fl, mean corpuscular Hb 22.8 pg, total white cell 4.9 × 10^9/l, and platelet 324 × 10^9/l. A hematological evaluation was performed due to RBC indices showing thalassemia (Table 1). Peripheral blood film examination revealed hypochromic microcytic RBCs, along with target cells, contracted RBCs, and stomatocytes. Hb analysis using high-performance liquid chromatography (HPLC) analysis showed a low hemoglobin A (Hb A) (55.1%), normal hemoglobin A2 (Hb A2) (3%), hemoglobin F (Hb F) (0.3%), and an additional peak at the C-window (33.4%) (Figure 1a). Quantitation of Hb using capillary electrophoresis (CE) also revealed a low Hb A level (66.4%), normal Hb A2 (3%), Hb F (0.1%), and an additional Hb C/Hb CS zone (30.3%).

Further testing on his parents revealed the following: the father's HPLC demonstrated multiple small peaks at retention times corresponding to Hb CS (4.6 and 4.9), whereas the mother's HPLC showed a peak in the C-window (36.5%), indicating Hb C. HPLC chromatograms of his father and mother are shown in Figures 1, b and c, respectively. His DNA analysis confirmed a termination codon mutation (TAA→CAA) in the alpha chain, identifying the Hb CS variant, and a heterozygous codon 6 (G>A) mutation in the beta chain, consistent with Hb C. These findings confirmed a diagnosis of thalassemia trait with the co-inheritance of Hb CS and Hb C.

Cascade screening revealed the mother as an Hb C carrier and the father as an Hb CS carrier. The patient remained active, with no anemia and no history of blood transfusions. Physical examination was unremarkable, with no hepatosplenomegaly. The hematological data of the patient and his parents are summarized in Table 1. Informed consent for publication was obtained from him. Ethical approval was obtained from the National Medical Research Registry (approval number: ID-24-02334-4U4).

Table 1. Summary of laboratory findings for patient and his parents

Parameters	Father	Mother	Patient	Reference range
CBC				
Hb (g/dl)	15.5	15.5	15.2	11.5–15.5
RBCs (× 10^{12}/l)	5.66	5.60	6.67	4.0–5.2
MCV (fl)	81.6	75.4	64.6	77.0–95.0
MCH (pg)	27.4	27.7	22.8	25.0–33.0
Hb quantification (HPLC)				
Hb A (%)	86.2	49.3	55.1	>90
Hb A2/E	2.7	3.3	3.0	-
Hb F (%)	0.6	0.4	0.3	<1
Others	Multiple small peaks at retention time ~4.6' and ~4.9'	C-window 36.5%	C-window 33.4%	-
Hb quantification (CE)				
Hb A (%)	97.4	61.9	66.4	>90
Hb A2	2.0	3.7	3.0	-
Hb F (%)	0.0	0.0	0.3	<1
Hb C	0.6	34.0	30.3	-
Molecular studies				
α-globin genotype	αα/α^{cs}α	αα/αα	αα/α^{cs}α	-
ß-globin genotype	Not done	ß/$ß^{C}$	ß/$ß^{C}$	-

CE=capillary electrophoresis; CBC=complete blood count; Hb=hemoglobin; Hb A=hemoglobin A; HbA2=hemoglobin A2; Hb C=hemoglobin C; Hb E=hemoglobin E; Hb F=hemoglobin F; HPLC=high-performance liquid chromatography; RBC=red blood cell; MCH=mean corpuscular hemoglobin; MCV=mean corpuscular volume

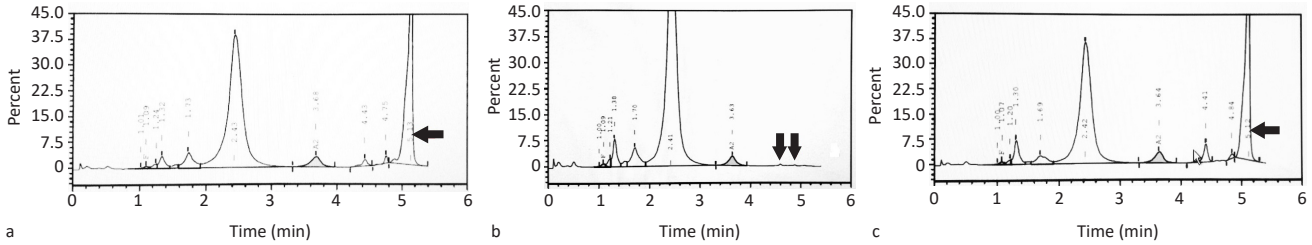

Figure 1. The high-performance liquid chromatography (HPLC) chromatograms results. (a) A peak in the C-window of the patient (black arrow); (b) multiple small peaks at retention times 4.6' and 4.9' of the father (black arrows); (c) A peak in the C-window of the mother (black arrow)

DISCUSSION

This case report highlights a patient with heterozygous Hb CS who is clinically asymptomatic and has almost normal RBC parameters,[4] as observed in his father. Mutated Hb is usually suspected when the Hb C/CS zone appears on CE or when a tiny peak is detected in the C-window with a retention time of 4.90–5.30 min on HPLC.[5] The Hb CS mutant allele was detected through DNA analysis using real-time polymerase chain reaction.[6] In contrast, patients with homozygous Hb CS exhibited hypochromic microcytic red cells, anisopoikilocytosis, leukoerythroblastic features, and basophilic stippling.[7]

Hb C is a common structural variant of normal Hb caused by the substitution of lysine for glutamate at the sixth position of the beta-globin chain.[2] It may manifest in homozygous (hemoglobin CC) or heterozygous (hemoglobin SC or hemoglobin AC) states. Individuals with Hb C trait are asymptomatic and have normal Hb levels. While some studies reported that MCV tends to fall within the lower end of the normal range, others observed microcytosis, often associated with concurrent iron deficiency and/or α-thalassemia traits, which are common within this demographic.[8] Despite being diagnosed with Hb C, his mother exhibited normal Hb levels and microcytosis, as indicated by red cell indices.

Our patient, with co-inheritance of Hb CS (alpha-thalassemia) and Hb C, showed normal Hb levels and microcytosis with decreased Hb C values. Forté et al[8] examined two infants with suspected Hb C trait/α-thalassemia who displayed microcytosis and low Hb C levels. A 12-year follow-up showed continued microcytosis and decreased Hb C values.[8] Similarly, another study revealed an MCV level of 66 fl and 32% of Hb C in three patients suspected of Hb C trait/α-thalassemia.[8] This combination can exacerbate microcytosis and depressed Hb C levels to a greater extent than either variant alone. The mechanisms underlying the suppression of Hb C synthesis in the presence of α-thalassemia remain unclear but may parallel those reducing hemoglobin S levels in sickle cell trait/α-thalassemia. These mechanisms potentially involve the influence of α chains on the control of globin synthesis, preferential binding of $ß^A$ over $ß^C$ chains to existing α chains, and relative instability of the mutant ß chain compared to its normal counterpart.[8]

In this case, he remained undiagnosed until the age of 7 due to the absence of clinical manifestations that led him to seek medical treatment. Studies on the co-inheritance of alpha-thalassemia and Hb C disease in various populations resulted in milder phenotypes, including lower MCV, higher Hb levels, and reduced risks of complications such as stroke, pain crises, infections, and the need for RBC transfusions. These findings align with the clinical presentation in this case.[8,9]

The limitation of this case is that Hb CS appeared in the C-window by HPLC alongside 10 other Hb variants (2 alpha and 9 beta variants). The most common variant in this window was Hb C, accounting for 92% of all cases. While Hb CS is the second most common variant in this window and has a similar retention time with Hb C, distinguishing between them when co-inherited is challenging due to the low percentage of Hb CS. This emphasizes the need for molecular studies for accurate identification. In this case, molecular studies played a crucial role in confirming the co-inheritance of Hb CS and Hb C, a diagnosis that might have been overlooked or misinterpreted through conventional hematological analyses alone. This case report provides insights into the hematologic profile of a patient with co-inheritance of Hb CS and Hb C, emphasizing the importance of comprehensive evaluation and management for this rare genetic

combination. Genetic counseling is essential for affected families to understand the inheritance patterns and risks for future offspring.[10]

Conflict of Interest

The authors affirm no conflict of interest in this study.

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