

CASE REPORT

Co-inheritance of compound heterozygous Hb Constant Spring and a single $-\alpha^{3.7}$ gene deletion with heterozygous $\delta\beta$ thalassaemia: A diagnostic challenge

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Abstract

Haemoglobin Constant Spring (Hb CS) mutation and single gene deletions are common underlying genetic abnormalities for alpha thalassaemias. Co-inheritance of deletional and non-deletional alpha (α) thalassaemias may result in various thalassaemia syndromes. Concomitant co-inheritance with beta (β) and delta (δ) gene abnormalities would result in improved clinical phenotype. We report here a 33-year-old male patient who was admitted with dengue haemorrhagic fever, with a background history of Grave's disease, incidentally noted to have mild hypochromic microcytic red cell indices. Physical examination revealed no thalassaemic features or hepatosplenomegaly. His full blood picture showed hypochromic microcytic red cells with normal haemoglobin (Hb) level. Quantitation of Hb using high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) revealed raised Hb F, normal Hb A₂ and Hb A levels. There was also small peak of Hb CS noted in CE. H inclusions was negative. Kleihauer test was positive with heterocellular distribution of Hb F among the red cells. DNA analysis for α globin gene mutations showed a single $-\alpha^{3.7}$ deletion and Hb CS mutation. These findings were suggestive of compound heterozygosity of Hb CS and a single $-\alpha^{3.7}$ deletion with a concomitant heterozygous $\delta\beta$ thalassaemia. Co-inheritance of Hb CS and a single $-\alpha^{3.7}$ deletion is expected to result at the very least in a clinical phenotype similar to that of two alpha genes deletion. However we demonstrate here a phenotypic modification of α thalassaemia presumptively as a result of co-inheritance with $\delta\beta$ chain abnormality as suggested by the high Hb F level.

Keywords: Hb Constant Spring, α^+ thalassaemia, $\delta\beta$ thalassaemia, capillary electrophoresis, molecular analysis

INTRODUCTION

Thalassaemias and hemoglobinopathies are the most common inherited disorders among humans, and they represent a major public health problem in many areas of the world, including South East Asia. The majority of α thalassaemia is caused by deletions that remove one ($-\alpha/\alpha\alpha$) or both ($--/\alpha\alpha$) of the two functional α globin genes leading to a decrease in α globin chain production. In Malaysia, a single $-\alpha^{3.7}$ deletion is common especially in the Malay population while the commonest non-deletional α thalassaemia is

Hb CS which is also seen mainly in the Malay population.¹ Interactions between the different determinants of α thalassaemia and Hb CS can produce a wide spectrum of clinical and hematological phenotypes, ranging from normal to the intermediate condition of thalassaemia. $\delta\beta$ thalassaemia results from the deletion of both δ and β genes, but with preservation of the gamma (γ) genes. The phenotype of heterozygotes resembles that of β thalassaemia trait, but Hb A₂ level is not increased. It is known that co-inheritance of both α thalassaemia and β thalassaemia would improve the phenotype

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and clinical symptoms of the patient because of alleviation of imbalance between α and non α chains.

The most common investigative tools used in the clinical laboratory for diagnosis of haemoglobinopathies and thalassaemia are alkaline and acid electrophoresis, and Hb A₂ and Hb F quantification by high performance liquid chromatography or capillary electrophoresis. The identification of Hb variants is often presumptive, based on characteristic electrophoretic mobility, quantity, and/or ethnic origin. Definite identification usually requires DNA analysis by polymerase chain reaction (PCR) or amino acid sequencing. A diagnostic assay to determine α thalassaemia mutation is important for genetic counseling and decisions for prenatal diagnosis. This case report highlights the diagnostic difficulty in a patient with compound heterozygosity of Hb Constant Spring and a single $-\alpha^{-3.7}$ deletion with heterozygous $\delta\beta$ thalassaemia.

CASE REPORT

This 33-year-old Chinese male patient was admitted to Universiti Kebangsaan Malaysia Medical Centre (UKMMC) initially due to dengue haemorrhagic fever. He was also known

to have Grave's disease and was on treatment. Physical examination revealed no thalassaemic features or hepatosplenomegaly. Examinations of other systems were unremarkable.

Laboratory findings

He was noted to have hypochromic microcytic red cell indices with normal Hb level. The haematological profile was: Hb 12.2 g/dL, RCC $6.15 \times 10^{12}/L$, MCV 63 fl, MCH 20.5 pg, MCHC 32.6 g/dl, RDW 18.3 %, platelet $25 \times 10^9/L$ and WBC $4.7 \times 10^9/L$. Peripheral blood film showed hypochromic microcytic red cells with mild anisopoikilocytosis. Iron status was normal (Iron 14.7 $\mu\text{mol}/L$; TIBC 51 $\mu\text{mol}/L$; ferritin 286.27 $\mu\text{g}/L$).

Hb analysis using cellulose acetate electrophoresis at alkaline pH showed a faint band at F region and high performance liquid chromatography (HPLC) analysis showed raised Hb F (10.8%) level with normal Hb A₂ (3 %) and Hb A (76.2 %) levels. Quantitation of Hb using capillary electrophoresis (CE) also revealed raised HbF (9.8 %); normal Hb A₂ (2.8 %) and Hb A (86.4 %) levels with additional Hb CS peak (Figure 1). H inclusions were negative. Kleihauer test was positive with heterocellular distribution of Hb F among the red cells.

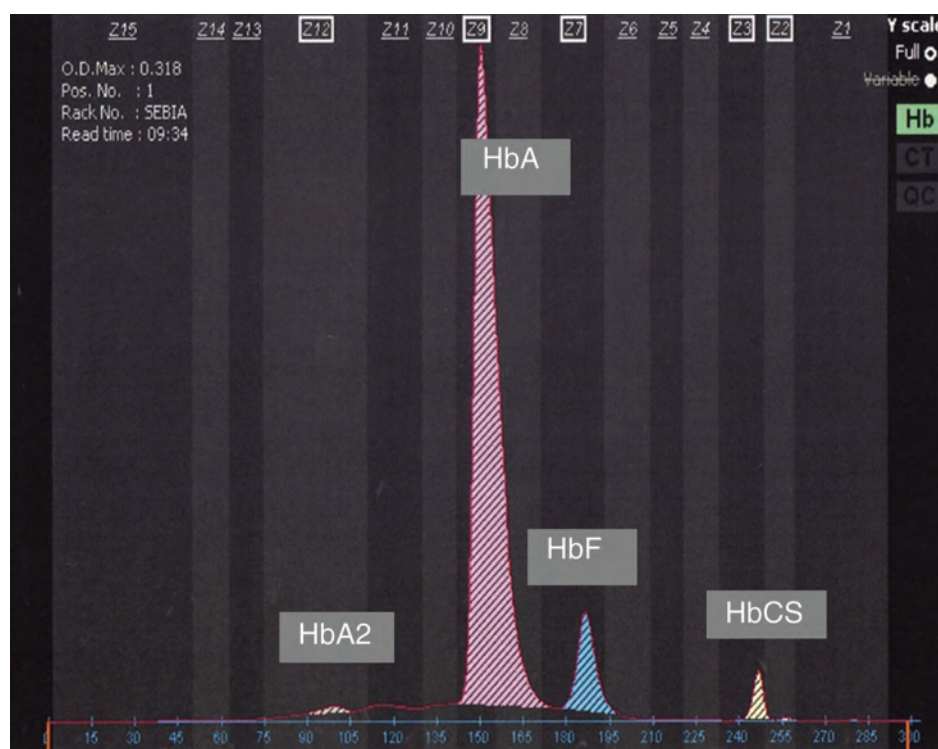


FIG. 1: Capillary electrophoresis analysis showing presence of Hb CS peak.

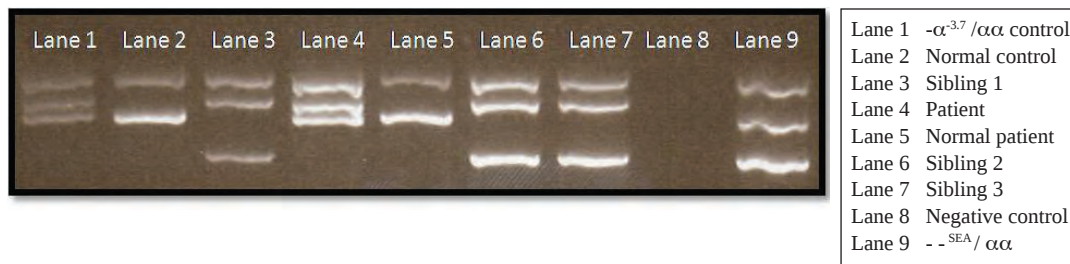


FIG. 2: DNA analysis by multiplex polymerase chain reaction of patient sample (lane 4) showed one α gene deletion (3.7 kb type) while his siblings (lane 3,6 and 7) were three α gene deletions.

DNA analysis using Multiplex-PCR amplification for α globin gene deletion showed a single $-\alpha^{3.7}$ deletion (Figure 2) and, real time PCR method for Hb CS mutation showed positivity for the mutant allele (MT) (Figure 3). These findings were suggestive of co-inheritance of compound heterozygous Hb CS and a single $-\alpha^{3.7}$ deletion with a concomitant heterozygous $\delta\beta$ thalassaemia.

Family profile

All his three siblings were well and no hepatosplenomegaly was noted. Their full blood picture showed mild hypochromic microcytic anaemia; and their Hb analysis showed normal Hb A₂ (1.4%) and Hb F (<1% - 1.8%) with presence of Hb H and Hb Bart's on cellulose acetate electrophoresis (Figure 4). DNA analysis showed all of them had α globin gene deletions

(Figure 2) but none carried Hb CS. Diagnoses of Hb H were made for all of them.

Unfortunately blood samples from his father was not available and his mother had passed away.

DISCUSSION

The patient described in this case report remained undiagnosed until the age of 30 years, as he apparently had no clinical manifestations that directed him to seek medical treatment. Interaction of α and β thalassaemia in this patient is believed to alleviate his phenotypic presentation. Many reports had discussed the co-inheritance of α and β thalassaemia, but to our knowledge there are no reported cases of co-inheritance of α and heterozygous $\delta\beta$ thalassaemia. These may be due to the non-functional significance of the δ thalassaemia,

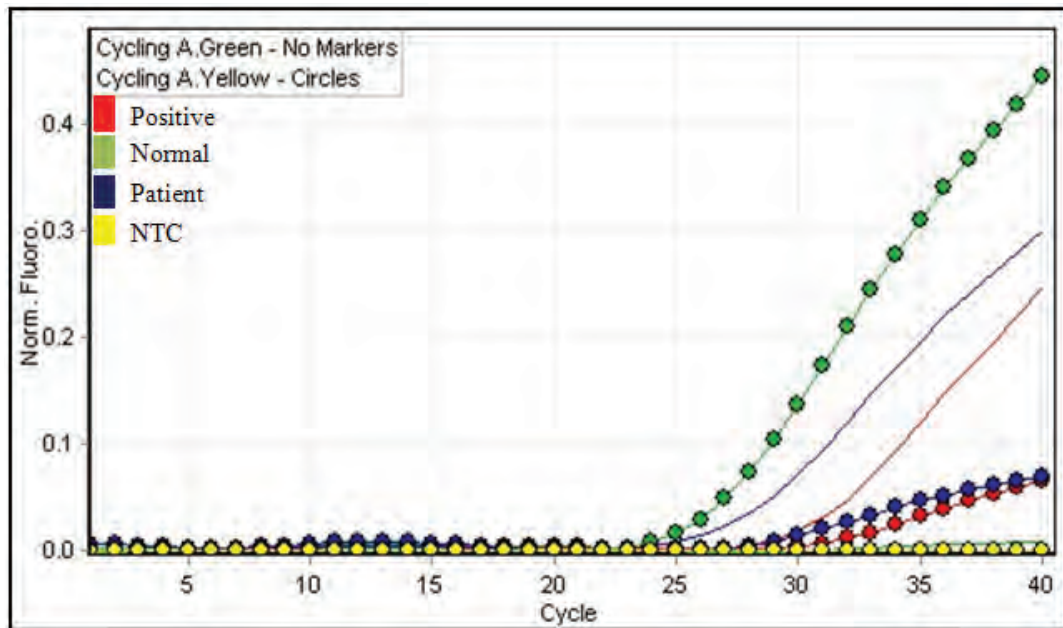


FIG. 3: DNA analysis by real time polymerase chain reaction showed presence of mutant allele (in blue) without wild type allele.

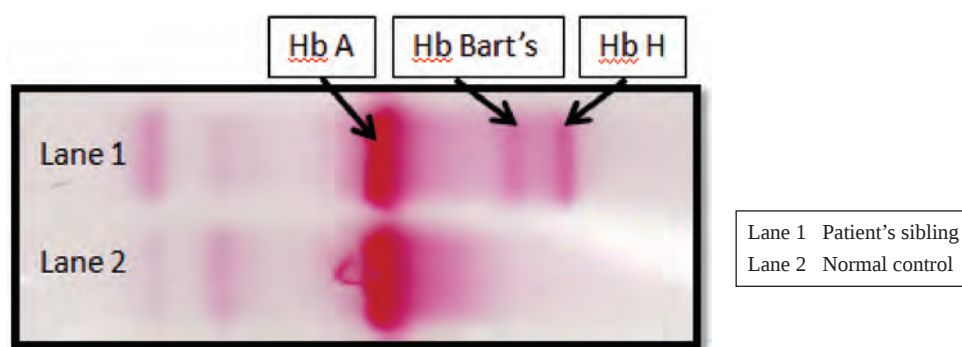


FIG. 4: Cellulose acetate electrophoresis at alkaline pH of one of patient's siblings showed presence of Hb Bart's and Hb H bands.

however their presence may complicate the diagnosis of β thalassaemia trait because of normalized Hb A₂ level.

In our case, a very faint band was seen at the F region of the alkaline cellulose acetate electrophoresis and quantification by HPLC and CE showed mild elevation of Hb F level and normal Hb A₂ level. β thalassaemia trait still cannot be totally excluded by normal Hb A₂ level, bearing in mind that Hb A₂ level can be normal in a patient with co-inheritance of α and β thalassaemia trait. However, Kleihauer test showed heterocellular Hb F distribution in the red cells which supports the diagnosis of heterozygous $\delta\beta$ thalassaemia in this patient.

DNA analysis for α globin gene deletion in this patient showed a single $-\alpha^{3.7}$ deletion. This deletion is present in a high frequency and in a wide distribution in Africa, Asia and the Mediterranean region. In Malaysia, the $-\alpha^{3.7}$ deletion was detected in all ethnic groups; Malays, Chinese and Indians.¹ In UKMMC, single $-\alpha^{3.7}$ deletion was the second commonest α thalassaemia trait after SEA deletion ($--^{SEA}$).² Out of 203 cases diagnosed as thalassaemia trait, 24.1% was $\alpha\alpha/-\alpha^{3.7}$ and majority were Malays. This was similar to a study done in UMMC by Wee *et al*,¹ where the majority of thalassaemia cases detected were $\alpha\alpha/-\alpha^{3.7}$ (64/103) and also mainly in Malays. In our laboratory, diagnosis of this gene deletion is using multiplex PCR amplification, in which the $-\alpha^{3.7}$ deletion sequence was amplified using $\alpha 2/3.7-F$ and $3.7/20.5-R$ primers. Deletion of this gene actually involves part of both alpha genes with a formation of a $\alpha 1\alpha 2$ fusion gene. This fusion gene is downregulated compared to a single $\alpha 2$ gene which in turn will be upregulated when the $\alpha 1$ is gene deleted, as in $-\alpha^{4.2}$ deletion. In our experience, the red cells indices in patients

with $-\alpha^{3.7}/\alpha\alpha$ were lower than those with $-\alpha^{4.2}/\alpha\alpha$ deletion.²

One particular common type of non-deletional α -thalassaemia present in Malaysia and other South-East Asian countries is Hb CS. Wee *et al*¹ found that the frequency of Hb CS in Malay population was 2.2% and it was not seen in Chinese and Indians. However, in UKMMC, few cases of Hb CS diagnosed were Chinese.²

Hb CS can be identified on cellulose acetate electrophoresis at alkaline pH, particularly if a heavy application is used and it moves between carbonic anhydrase enzyme and Hb A₂ while in HPLC it appears in the C window.³ Surprisingly, the above findings were not seen in our patient's blood sample. However, presence of Hb CS was noted in CE whereby the peak of this Hb was detected at zone 3 (Figure 1) with a percentage of 0.2%. A study by Waneesorn *et al*⁴ showed that the CE method was superior to HPLC method in detecting Hb CS especially in heterozygote state. Another study done by Liao C *et al*⁵ reported that the CE method was suitable for the routine screening of Hb CS trait as it could quantify Hb CS level as low as 0.1%. Our finding was further supported by the detection of Hb CS mutant alleles through DNA analysis using real time PCR method (Figure 3). In our laboratory, amplification of the mutant allele for Hb CS and normal $\alpha 2$ allele (wild type) was carried out using allele-specific fluorescence PCR. However, in this patient, the real time PCR finding showed no wild type allele. Since this patient also has $-\alpha^{3.7}$ deletion, this may explain why the wild type allele was not amplified.

Tangvarasitticha O *et al*⁶ has looked into the efficiency of different diagnostic methods in detecting Hb CS by using mutation specific restriction enzyme digestion (RED), amplification refractory mutation system

(ARMS) and automated HPLC. The results demonstrate that the DNA-based methods, RED and ARMS, are efficient diagnostic tools in detecting HbCS in homozygotes and heterozygotes, whereas automated HPLC gave accurate results for homozygous Hb CS, but missed some cases of Hb CS trait. Standard haematological methods (determination of mean cell hemoglobin and mean cell volume) did not efficiently differentiate homozygous Hb CS and Hb CS trait from samples with normal α globin chains. Cases unresolved by ARMS should be clarified by appropriate methods such as DNA sequencing.

Real-time PCRs with SYBR Green1 and ABI7000 (SYBR-PCR) followed by dissociation curve (DC) analysis were used to detect the —SEA, — $\alpha^{3,7}$, — $\alpha^{4,2}$, and non-deletion-type alleles, respectively and has been shown to improve the detection of co-inheritance of the above mentioned conditions.⁷

$\delta\beta$ thalassaemia is a relatively rare form of thalassaemia, characterized by absence of β and δ globin production. The heterozygotes for this syndrome have a thalassaemia red cell indices, normal or reduced Hb A2 levels and increased amounts of fetal hemoglobin which is heterogeneously distributed among the red cells.⁸ Hereditary persistent fetal haemoglobin (HPFH) needs to be differentiated from $\delta\beta$ thalassaemia as both conditions have elevated level of Hb F. The distinction between these conditions is subtle and is made on clinical and hematological grounds. HPFH includes a wide range of conditions, but in heterozygotes it is typically characterized by levels of Hb F of up to 30% with normal red cell indices. On the other hand heterozygotes for $\delta\beta$ thalassaemia tend to have less elevation of Hb F levels (5% to 20%) accompanied by

hypochromic, microcytic red cell indices.^{9, 10}

It is important to differentiate $\delta\beta$ thalassaemia with HPFH for genetic counselling because compound heterozygotes for HPFH and β thalassaemia have a very silent or mild phenotype in contrast to combination of $\delta\beta$ thalassaemia and β thalassaemia which may result in β thalassaemia major. The distinction may be made by analyzing the red blood cell distribution of Hb F. Hb F is usually heterogeneously distributed in the $\delta\beta$ thalassaemia trait in contrast to HPFH in which it is homogeneously distributed. However, a definitive diagnosis can only be made by identification of the deleted mutation by DNA analysis.¹¹

Co-inheritance of α thalassaemia and $\delta\beta$ thalassaemia is assumed to have some alleviation to the phenotypic presentation of our patient. Li *et al*¹² reported two cases of $\delta\beta/\beta$ thalassaemia whereby one of the patient also co-inherited α thalassaemia. $\delta\beta/\beta$ thalassaemia often presented as thalassaemia intermedia, however the patient without a co-inheritance of α thalassaemia presented with more severe anaemia (Hb 5.9 g/dl, transfusion occasionally are imperative).

All patient's siblings showed drastic haematologic parameters improvement with no clinical features of thalassaemia intermedia such as hepatosplenomegaly. One of his siblings showed almost normal Hb level (11.1g/dl) (Table 1) which was very unusual in a patient with Hb H. In our previous study² we found that mean Hb level for Hb H patient was 8.7g/dl. His siblings also showed negative Kleihauer tests, however their Hb analysis showed presence of Hb Bart's. Adult Hb H patients usually do not show presence of Hb Bart's. This indicate presence of excess γ globin chain production secondary to up regulation of γ gene which is most likely due to

TABLE 1: Summary of laboratory findings for patient and his siblings

Case no.	Patient	Sibling 1	Sibling 2	Sibling 3
Age (yr) /Sex	33/male	32/female	30/male	24/female
Hb g/dL	12.2	9.0	11.1	10.4
RBC x 10 ¹² /L	6.15	4.71	5.41	4.84
MCV fl	63.0	61.5	66.0	65.2
MCH pg	20.5	19.2	20.6	21.5
Hb A2 (%)	3	1.4	1.4	1.4
Hb F (%)	10.8	1.8	<1%	<1%
Hb H	none	present	present	present
Hb Barts	none	present	present	present
Kleihauer test	positive	negative	negative	negative
Genotype	$\alpha\alpha\text{CS}/\alpha^{-3,7}$	$--_{\text{SEA}}/\alpha^{-3,7}$	$--_{\text{SEA}}/\alpha^{-3,7}$	$--_{\text{SEA}}/\alpha^{-3,7}$

the inheritance of a $\delta\beta$ gene mutation together with the three α gene deletions. However in this patient there are only two α genes involved, thus enough to cause raise in Hb F.

This case has been a diagnostic challenge to our laboratory, even though our patient is asymptomatic. It is important to identify this patient's genetic condition because thalassaemia is common in our part of the world and genetic counselling is important for our patient as he has a high possibility of having an offspring with Hb H disease which may be present with thalassaemia intermedia phenotypic features. Therefore a combination of conventional method (gel electrophoresis, capillary electrophoresis, HPLC) and advanced method (DNA analysis) with good and accurate interpretations are imperative in order to make a correct diagnosis for thalassaemia.

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