

CASE REPORT

IDENTIFICATION OF HEMOGLOBIN AC HETEROZYGOTE STATUS IN A MALAY FAMILY: A DECISION BETWEEN HEMOGLOBIN ELECTROPHORESIS AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract. Thalassemia is a common public health problem among Malays. Hemoglobin C (Hb C) is a hemoglobin beta variant resulting from a single base mutation at the 6th position of the β -globin gene leading to the substitution of glycine for glutamic acid. Hb C is commonly detected in West Africans and in African American but has not been reported in Malaysia. It can be falsely diagnosed as HbE trait in the Malaysian Thalassemia Screening Program which utilizes cellulose acetate hemoglobin electrophoresis. This is the first reported case of Hb AC heterozygote status in a Malay family, with unusual splenomegaly in one of the family members.

INTRODUCTION

Thalassemia is a common inherited red cell disorder among Malays with frequencies of 20% for α -thalassemia, 3-50% for Hb E, 3-4% for β -thalassemia, and 1-4% for Hb Consant Spring (George and Khuziah, 1984). One form of the β variant is Hb C which results from a single base mutation of the β globin gene at position 6 leading to substitution of glycine for glutamic acid. To date there was no Hb C reported in Malaysia. In most hospitals in Malaysia, hemoglobinopathies are diagnosed by hemoglobin electrophoresis in an alkaline pH, in which Hb C, Hb O-Arab, Hb Malay and Hb E are found in a single position within one band. This technique does not dif-

ferentiate these different types of Hb potentially contained within that band. They can only be reliably differentiated by high performance liquid chromatography (HPLC) (Joutovsky and Nardi, 2004). The high sensitivity and specificity of HPLC allows proper resolution of the single electrophoretic band and can differentiate some encountered hemoglobin variants, such as E and Lepore from A, and D from G (Ou and Rognerud, 2001).

We report a Malay family diagnosed with heterozygous Hb AC based on the findings of HPLC and electrophoretic analyses.

CASE REPORT

A 9-year-old girl was referred to Hospital-USM with complaints abdominal distension for few months due to her increasing spleen size. She had no abdominal pain, and no associated symptoms of fever, malaise, nausea or vomiting. She had no history of hemoglobinuria or jaundice. On examination, she was

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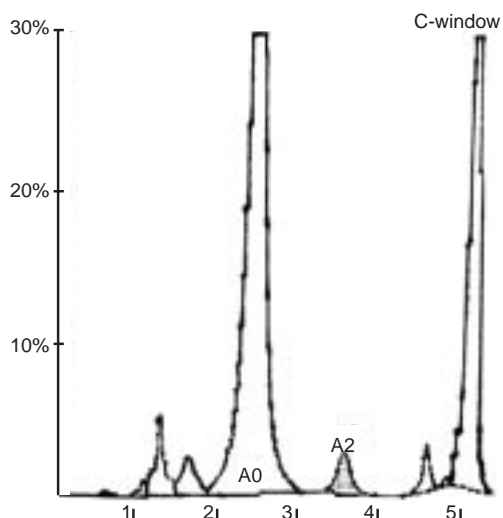


Fig 1—High performance liquid chromatography (HPLC) of hemoglobin in a patient with heterozygote hemoglobin AC status. Note the peak in the C-window with a retention time of 5.13 minutes.

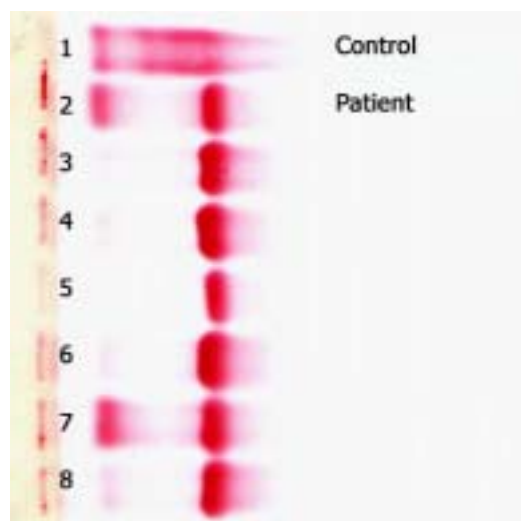


Fig 2—Cellulose acetate hemoglobin electrophoresis. Lane two shows the results from the patient.

pink, afebrile, with no jaundice. The spleen measured 8 cm below the costal margin. Her Hb was 11.4 g/dl, the total white blood cell count was $3.5 \times 10^9/l$, and the platelet count was $63 \times 10^9/l$. The mean corpuscular volume (MCV) and mean cell hemoglobin (MCH) were 74.4 fl and 25.2 pg, respectively. Hypochro-

mic microcytic red blood cells with numerous target cells were detected on the peripheral blood film. Her Hb types and relative quantities were evaluated by an HPLC automated hemoglobin analyzer (Variant Testing System: Bio-Rad Laboratories, Hercules, CA). She had a peak at the C-window with a retention time of 5.13 minutes, with the presence of A and A2 (Fig 1). Hb analysis using cellulose acetate Hb electrophoresis at a pH of 8.6 exhibited a Hb E band (Fig 2), whereas acid citrate agar electrophoresis at a pH of 6.2 showed a C-band. There was no evidence of hemolysis on the hemolytic work-up performed. A diagnosis of Hb AC heterozygote status was made. Screening of other family members showed the mother and two of four other siblings had Hb C trait. The Hb AC positive family members had normal Hb, MCV and MCH, and none had a palpable spleen (Table 1).

DISCUSSION

Hemoglobin C trait is a rare type of hemoglobinopathy which has not been described in the Malay population previously. Hb C is due to a single base mutation at codon 6 of the β globin gene leading to substitution of lysine for glutamic acid ($\alpha 2\beta 26^{Glu-Lys}$). The electrophoretic detection of Hb C may be misleading as it runs parallel with Hb E and A2 in cellulose acetate hemoglobin electrophoresis (Ou and Rognerud, 2001). Furthermore, Hb E trait and disease are the most prevalent types of hemoglobinopathies in the Malay population with an incidence ranging from 3 to 50% in West Malaysia (George and Khuziah, 1984).

The necessity for further investigations in this patient emerged due to a number of reasons: she presented with moderate splenomegaly which is rare for Hb C trait. The level of the A2 band was higher than expected for Hb E trait, except in the case of co-existence of β -thalassemia. Moreover, the reported family is originally from the Malaysian state of Kelantan

Table 1
Hematological parameters in a family with heterozygous hemoglobin AC status.

	Father 47 years	Mother 45 years	Son 23 years	Daughter 21 years	Son 12 years	Patient 9 years	Daughter 4 years
Hb (g/l)	149	130	167	149	138	114	112
MCV (fl)	81.1	85.0	87.2	81.1	84.8	74.4	73.7
MCH (pg)	27.5	28.0	29.9	28.0	29.3	25.2	24.7
Hb A (%)	84.1	52.0	51.6	52.2	85.7	53.5	84.5
Hb A2 (%)	3.1	3.5	3.5	3.4	3.56	3.6	3.2
Hb C (%)	-	36.6	37	36.8	-	35.3	-
HPLC (retention time for c-window)	-	W(5.13)	W(5.13)	W(5.14)	-	W(5.13)	-

in Northeast Malaysia, close to the southern Thai border. In Thailand, Hb E and α -thalassemia make up 4-53% and 15-75% of the Thai population, respectively (Fucharoen *et al*, 2004). Some 15 cases of Hb C have been reported, of which 3 were Hb EC compound heterozygotes (Sanchaisuriya *et al*, 2001).

The patient in this case presented with moderate splenomegaly and pancytopenia with no underlying hemolysis. Splenomegaly could possibly be due to co-infection with malaria not detected during our investigations. Hb C probably did not influence the enlargement of the spleen. Splenomegaly was not detected in other family members; and is an unusual finding in Hb C trait (Adekile *et al*, 1993).

In most hospitals in Malaysia, cellulose acetate Hb electrophoresis at an alkaline pH is used as a screening procedure for thalassemia. Citrate agar in acid pH is only used in cases suspected of having sickle cell disease, whereas it may also enable the differentiation between Hb C from O-Arab, C-Harlem and E (Anonymous, 1998). HPLC is not routinely used in all hospitals. Hence, it is recommended that it should be introduced into routine practice.

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