CASE REPORT

Secondary polycythaemia in a Malay girl with homozygous Hb Tak

Habizah Salwa AMRAN*,** MBBC,HBao, Mimi Azura AZIZ* MD, MPath, Elizabeth GEORGE** MBBS, FRCPE, Noraeah MAHMUD* MBBS, MPath, Tze Yan LEE** BSc, MSc and Sabariah MD NOOR** MD, MPath

Abstract

Habizah Tak is one of more than 200 high affinity haemoglobin variants reported worldwide. It results from the insertion of two nucleotides (AC) at the termination codon, between codon 146 and codon 147 of the beta-globin gene [Beta 147 (+AC)]. Polycythaemia is the main clinical feature although affected carriers are usually asymptomatic and do not require intervention. Several case studies in this region have reported the co-inheritance of Hb Tak with Hb E, delta beta and beta thalassaemia with one case of homozygous Hb Tak in a Thai boy. In this case report, a cluster of haemoglobin Tak was found in a family of Malay ethnic origin. Cascade family screening was conducted while investigating a 4-year old girl who presented with symptomatic polycythaemia. She had 2 previous Hb analysis done, at 7-month and 2-year-old with the diagnosis of possible Hb Q Thailand and Homozygous Hb D, respectively. Both diagnosis did not fit her clinical presentations. She was plethoric, had reduced exercise tolerance as well as cardiomyopathy. Her parents were consanguineously married and later diagnosed as asymptomatic carriers of Hb Tak. Consequently, re-analysis of the girl’s blood sample revealed a homozygous state of Hb Tak. In conclusion, high oxygen affinity haemoglobin like Hb Tak should be considered in the investigation of polycythaemic patients with abnormal Hb analyses. In this case, DNA analysis was crucial in determining the correct diagnosis.

Keywords: Hb Tak, high oxygen affinity haemoglobin variant, polycythaemia

INTRODUCTION

Habizah Tak is a Hb variant that results from the insertion of two nucleotides (AC) at the termination codon between codon 146 and 147 of the beta-globin gene. The insertion causes a frameshift in the terminating codon 147 leading to an elongated beta chain by 11 amino acids. The main property of this haemoglobin is its high affinity towards oxygen binding. The purified Hb Tak has a very low P50 value and is not influenced by the addition of 2,3 diphosphoglycerate. Its oxygen dissociation curve is shifted to the left compared with the normal red cells showing its high oxygen affinity.

Heterozygous Hb Tak has 30-40% of Hb Tak with normal or slightly elevated Hb levels between 13-17 g/dL. Clinically, they are asymptomatic and are detected incidentally during a population screening programme or a family study with a known affected patient.

A large cohort study was carried out in Thailand to look at the various haemoglobin variants present in their population. Hb Tak was found in 14.2% of the 636 positive cases of haemoglobin variants. The majority of these cases were from central and northeast Thailand. Heterozygous and compound heterozygous Hb Tak with other thalassaemia or haemoglobin variant have been described in Malaysia, Taiwan and Hong Kong. The Malaysian case was reported in 1977, and was a heterozygous Hb Tak in a 4-day-old newborn Malay boy who suffered from severe neonatal jaundice. Structural study and amino acids analysis was carried out to identify the abnormal haemoglobin. The haemoglobin had an elongation of the beta globin by 11 residues at the C-terminus which was similar to the structure of Hb Tak. Our case report describes the first homozygous Hb Tak found in a Malay girl.
CASE REPORT

Background
The patient was investigated following a cascade screening for haemoglobinopathy. The index case, NF, was a 4-year-old girl who was referred by the National Heart Institute Kuala Lumpur to the Genetic Clinic, Hospital Kuala Lumpur, for investigation of possible genetic conditions associated with cardiomyopathy in the paediatrics age-group.

Earlier in another centre, she was diagnosed to have homozygous haemoglobin D, but no DNA analysis was done to confirm this. Her parents’ blood samples were taken and Hb analysis show discrepancies between the patient and her parents’ results. Thus, new samples were requested from the family to confirm the diagnosis by DNA sequencing.

History and clinical presentation
NF was the only child of consanguineous parents, who was born at term via spontaneous vaginal delivery at a local hospital in Malaysia. Her birth weight was 2.5 kg and her mother had an uneventful antenatal history. At 7 months of age, she was admitted for bronchopneumonia associated with jaundice. Her haemoglobin level was 9.0 g/dL and the peripheral blood film test showed features of haemolysis. Haemoglobin analysis was sent to the General Hospital Kuala Lumpur. The report suggested possible Hb Q Thailand with co-inheritance of alpha or other beta variants. Thus, DNA analysis was suggested but unfortunately, the family migrated to another state and defaulted follow up.

During this time, she was also seen at the National Heart Institute Kuala Lumpur for investigations of a heart murmur. She was noted to have a patent foramen ovale with left to right shunt and a mild mitral regurgitation. At 2 years old, she had a second haemoglobin analysis done at another health centre in Kuala Lumpur but this time, a diagnosis of homozygous Hb D was made. Similarly, no confirmatory DNA analysis was carried out.

Throughout her early childhood, she required frequent clinic visits and hospital admissions for respiratory tract infections. She had one intensive care unit admission for severe pneumonia. Fortunately, no mechanical ventilation was required.

At her new residence in Johor Bharu, she was noted to have persistent symptomatic polycythaemia and was seen by paediatricians at the Hospital Sultanah Aminah, Johor Bharu. Therapeutic venesection was carried out every 3-4 weeks to relief her symptoms, with good effect.

Currently, she is 4 years old and her growth chart is below the 10th centile with frail, plethoric features. On examination, she has a soft pansystolic murmur with no hepatosplenomegaly. Her haemoglobin levels ranged between 20 to 25 g/dL, depending on the last venesection done. Her echocardiogram showed dilated ventricles, moderate tricuspid regurgitation with pressure gradient of 48 mmHg, mild mitral regurgitation, and good ejection fraction at 70%.

Her current treatment consists of syrup Furosamide, Aldactone and Captopril, as well as regular venesection. Developmentally, her speech and language are normal, but her gross motor skills are limited due to reduce exercise tolerance. She is able to recognise numbers and letters, and holds a pencil in a mature grip. Her gait is normal and neurological examination including cranial nerves are intact.

She had never received any exchanged transfusion and the DNA analysis for congenital heart disease associated with cardiomyopathy screening were negative.

Laboratory investigations
Patient and parents blood samples were collected in EDTA bottle and subjected to complete blood counts (CBC), blood smear, haemoglobin analysis and capillary gel electrophoresis at pH 8.5 and pH 6.0.

The child’s CBC showed haemoglobin of 24.3 g/dL with erythrocytosis, normochromic normocytic red cells and mild thrombocytopenia of 104 X 10^9/L. The blood smears showed some platelet clumps and satelitism that may explain the low count in the automated haematology analyser. The father also had polycythaemia with haemoglobin level of 18.8 g/dL whereas the mother, being in her reproductive age, had normal haemoglobin at 14.0 g/dL. Other red cell parameters for the parents were within the normal limits.

High performance liquid chromatography (HPLC) of the index case NF, showed mildly increased Hb F and Hb A2 levels. A variant haemoglobin eluted at RT 4.08 (D-window) measuring at 88.3%. The parents had normal Hb F and HbA2 levels and reduced Hb A level. The Hb variant level for father and mother was 32.3% and 35% respectively.

Further analysis by capillary electrophoresis
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(CE) showed the index case had mildly raised Hb A2 with 92.1% Hb variant at zone 7. Her parents had reduced Hb A with Hb variant level of 32.8% and 34.4% respectively (Table 1 and Fig. 1).

Haemoglobin gel electrophoresis (GEP) tests of the child showed an abnormal band between Hb S/D/G and Hb F region in alkaline pH 8.5, while in the acid gel pH 6.0 the abnormal band was anodal to the Hb A region (Fig. 2).

DNA sequencing confirmed the presence of an AC dinucleotide into 147 region of the beta globin gene. The child (proband) was homozygous for this mutation while the parents were carriers (Fig. 3).

DISCUSSION

Haemoglobin Tak was reported in various regions in Asia in forms of heterozygous and compound heterozygous inheritance but a homozygous state was only reported in a Thai boy in 2003. This is the first case report of homozygous Hb Tak in a Malay girl who presented with symptomatic polycythaemia and cardiomyopathy.

The clinical presentation of Hb Tak depends on the amount of the high oxygen affinity haemoglobin produced compared to the normal adult haemoglobin, Hb A. As expected, the patient with homozygous Hb Tak have higher red blood cells count with prominent hyperviscosity symptoms like headache and recurrent chest infection due to sluggish respiratory blood flow. Heterozygous patients on the other hand, would have no apparent symptoms with normal to mildly increased red cell counts.

The index case who is a homozygous Hb Tak has hyperactive myocardium with dilated ventricles. This may be due to the increase in hypoxic drive as Hb Tak is a high oxygen affinity haemoglobin, thus reducing release of oxygen to the tissues. The associated secondary erythrocytosis is another mechanism to compensate the tissue hypoxia.

Generalised growth failure as well as poor exercise tolerance in this girl was also seen in the Thai boy with homozygous Hb Tak. Her parents being heterozygous, did not experience any clinical symptoms related to the secondary erythrocytosis.

The complete blood count and the blood smear for the index case with homozygous Hb Tak showed polycythaemia with marked erythrocytosis. Her father also had polycythaemia but was clinically asymptomatic. On the other hand, her mother had normal haemoglobin and red cell counts. These may be attributed to her monthly menstrual cycle and thus no significant polycythaemia on the blood count indices were observed.

![FIG. 1: (A) HPLC and (B) CE of index case](image)

TABLE 1: HPLC and CE of index case and parents

<table>
<thead>
<tr>
<th></th>
<th>Hb A (%)</th>
<th>Hb A2 (%)</th>
<th>Hb F (%)</th>
<th>Hb Variant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPLC</td>
<td>CE</td>
<td>HPLC</td>
<td>CE</td>
</tr>
<tr>
<td>Child, NF (index)</td>
<td>-</td>
<td>-</td>
<td>4.4</td>
<td>4.7</td>
</tr>
<tr>
<td>Father</td>
<td>57.7</td>
<td>63.3</td>
<td>2.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Mother</td>
<td>55.4</td>
<td>61.9</td>
<td>2.5</td>
<td>3.7</td>
</tr>
</tbody>
</table>

(A) (B)
Compound heterozygous delta beta/Hb Tak and $\beta^0$/Hb Tak caused similar phenotypes to homozygous Hb Tak due to the absence of a normal beta globin gene effect.\(^1,3,7\) However, these cases present later in young adulthood with plethoric appearance and erythrocytosis. A case report on compound heterozygous Hb $\beta^0$/ Tak in Hong Kong described a young male presenting with erythrocytosis and splenomegaly. He was initially diagnosed as polycythemia rubra vera (PRV) but the other typical peripheral blood and marrow findings of PRV were lacking.\(^3\) In this case, the diagnosis of PRV was made due to the markedly increased normochromic

FIG. 2: GEP of index case in (A) alkaline pH 8.5 and (B) acid pH 6.0 gels

FIG. 3: DNA sequencing of index case (proband) & parents which showing Beta 147 (+AC)
normocytic red cells and raised haematocrit level. The subsequent Hb analysis and DNA sequencing confirmed the diagnosis of compound heterozygous Hb β⁰/δ⁰ Tak.

In compound heterozygous delta beta/ Hb Tak, β⁰/Hb Tak or homozygous Hb Tak with concomitant alpha thalassaemia, instead of normochromic normocytic red cells, red cell microcytosis may be present.¹³

The index case was erroneously diagnosed as Hb Q Thailand and Hb D disease at 2 different occasions as the haemoglobin analysis show presence of haemoglobin variant at D window in HPLC. Hb Tak elutes at D window (RT 3.98-4.31) or S window (RT 4.31-4.38) in HPLC.⁸ Both Hb Tak and Hb Q Thailand were seen to be the most common haemoglobin variant in S-window in a Thai study.¹⁰ However, Hb Q Thailand and Hb D disease did not match the clinical presentation of polycythaemia.

Additional analysis with capillary electrophoresis (CE) or gel electrophoresis (GEP) is recommended to investigate further the haemoglobin variant. Haemoglobin Tak appears at zone 7 in capillary electrophoresis.¹¹ In alkaline GEP it is seen between Hb S/D/G and Hb F region and moves anodal to Hb A in acid.³⁹

These methods should be able to exclude Hb S and Hb D, followed by confirmation by DNA sequencing. In centres where Hb Tak oligonucleotides primers are available, allele specific PCR may be used to confirm the diagnosis, for example the use of multiplex allele specific PCR by the Thai investigators to simultaneously detect the presence of Hb Tak, Hb S and Hb D Punjab.¹²

Treatment options recommended for homozygous Hb Tak are exchange transfusion and allogenic stem cell transplant if a suitable donor is available. At present, this girl is on regular venesection and exchange transfusion has not been started. Exchange transfusion is a preferred method than venesection as the exchanged RBCs have normal oxygen affinity thus, reducing the hypoxic tissue drive. This will subsequently improve her compensatory erythrocytosis. In her case, allogenic stem cells transplant was not a likely option, as she is an only child and no potential donor is available.

In conclusion, careful interpretation of haemoglobin analysis, as well as its correlation with clinical history is important in determining the correct diagnosis of haemoglobinopathy. This is especially in the paediatrics age group and young adults with secondary erythrocytosis, where an abnormal Hb analysis should be followed by confirmatory DNA analysis as the presence of a high affinity oxygen haemoglobin should be considered.

ACKNOWLEDGEMENT

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REFERENCES


