A Rare Case of Alpha-Thalassaemia Intermedia in a Malay Patient Double Heterozygous for $\alpha^+$-Thalassaemia and a Mutation in $\alpha_1$ Globin Gene CD59 (GGC→GAC)

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INTRODUCTION
The $\alpha$-thalassemias are the most common single-gene diseases in the world. They are characterized by a reduction or complete absence of $\alpha$-globin gene expression. Normal individuals have two $\alpha$ genes on each chromosome 16 ($\alpha\alpha/\alpha\alpha$). The loss of one ($-\alpha$) or both ($-\alpha\alpha$) of the cis-linked genes are the most common causes for $\alpha$-thalassemias. Patients with alpha-thalassaemia intermedia with only one functional $\alpha$-globin gene ($-\alpha/\alpha\alpha$) develop chronic haemolytic anaemia of variable severity. HbH disease is the common cause alpha-thalassaemia intermedia. This condition is characterized by a strongly positive H-inclusion test. Non-deletional HbH disease has been described to be more severe than the deletional type. The most severe form of alpha thalassaemia is Hb Barts hydrops foetalis where there are no functional $\alpha$-globin genes ($-\alpha/\alpha$) and results in the condition where death occurs in utero or within a few hours of birth. In this case report, a rare case of alpha-thalassaemia intermedia is presented where the H-inclusion test is negative.

CASE REPORT
A 52-year old Malay male presented with pallor, generalized malaise, jaundice and hepatosplenomegaly. There was no family history of thalassaemia. The haematology work-up included a full blood picture, thalassaemia screen tests and a liver profile. Blood specimens were drawn into tubes (Becton Dickinson Vacutainer Systems) containing dipotassium ethylene diamine tetraacetic acid (EDTA) for full blood picture and thalassaemia diagnosis (quantification of Hb subtypes and DNA studies), into plain tubes for serum ferritin and liver profile studies.

Thalassaemia screen using the BHES protocol was done. The acronym BHES refers to a multi-step process for screening of thalassaemia where B is for blood counts and blood film, H for high performance liquid chromatography, E for electrophoresis and S for stability. B: Blood counts were generated on an automated blood counter (Cell-Dyn, Abott laboratories). He was noted to have severe anaemia with a haemoglobin level of 5.6 gm/dl (normal range 13-18 gm/dl), hypochromic-microcytic red cell indices, reticulocytosis and a thalassaemia peripheral blood film (anisopoikilocytosis, hypochromia, basophilic stippling, target cells, and fragments). H: a specimen collected in EDTA was analyzed on the Bio-Rad Variant high performance liquid chromatography (HPLC) Hb analyzer (Bio-Rad Laboratories) to determine the distribution of Hb subtypes with the use of the $\beta$-thalassaemia short program recorder pack as described in the instruction manual for the assay. The Hb subtypes noted were HbA, HbF, HbA and a pre-run peak of Hb Barts, shown Figure 1. There was no HbH seen. E: Hb electrophoresis by automated agarose gel electrophoresis (Sebia) on alkaline pH8.5 showed A=prerun peak of Hb Barts, RT=retention time in minutes Y axis: percentage of Hb subtypes, X axis: retention time (RT)

Fig. 1: HPLC C-gram of alpha thalassaemia-intermedia (CD59/-$\alpha^+$)
similar findings of Hb subtypes seen with HPLC. S: the H-
inclusion test was negative. The serum ferritin was 340µg/L.
(normal 150-300 µg/L). The liver enzymes were normal and
the unconjugated bilirubin level was raised. The antiglobulin
test (Coombs test) was negative. Genomic DNA was extracted
from peripheral blood using standard methods and multiplex
polymerase chain reaction (PCR) was done to detect the
following -thalassaemia mutations: single deletion (-α+),
-α+), two gene deletions (-α3.7,-α2.8), three gene deletions
(-α3.7,-α2.8,-α2.0), and non-deletion -α-thalassaemia (initiation codon (CD) (GTG-GTG),
CD30, CD35 (TCC-CGC), Hb Quong Sze or CD125
(CTG-CCG), Hb Constant Spring or CD 142 (TAA-CAA) and,
CD59 (GGC-GAC). The patient was found to be
heterozygous for the single deletion (-α+) and the non-
deletion mutation CD59 (GGC-GAC).

DISCUSSION
Patients with alpha thalassaemia-intermedia have moderate
anaemia with Hb levels between 7-10gm/dl. The manifestations
include thalassaemia facies, jaundice and hepatosplenogalaly that range from mild to moderate. Growth
and development is normal. Gallstones and iron overloading
in the absence of blood transfusions have been seen. The H-
inclusion test is strongly positive in these cases. In this case
report, the patient had -α-thalassaemia intermedia phenotype
with severe anaemia (Hb 5.6 gm/dl) with thalassaemia
features in the peripheral blood film in the absence of a
positive H-inclusion test.

Alpha-thalassaemia is caused by both deletion and non-
deletion mutations in the alpha globin gene complex. In contrast to β-thalassaemia, deletions are more common than
non-deletional defects. Commonly, -α-thalassaemia-
intermedia presents as HbH disease from the interaction of
deletion of both the α-globin genes in cis (αα) (thal 1) and αα
(αthal 2) phenotype. The molecular defects resulting in a
single deletion of the α globin gene are the rightward deletion
-αααα and the leftward deletion -αααα with -αααα being the more
common mutation. The double deletion defects (αααα of the α
globin genes in cis are -α3.7, -α2.8, -α3.7, -α2.0 and -α2.0). The most
common non-deletion single gene defect (αα) is Hb Constant
Spring. This is a termination codon defect (TAA-CAA) results
in a long mRNA with 31 extra amino acids being formed. The
long mRNA is unstable resulting in reduced production of α
globin chains and a αα phenotype. HbH-Constant Spring has
a more severe clinical phenotype than deletion HbH disease.
The nondeletion molecular defect in the α-globin 1 gene
CD59 (GGC-GAC) results in the formation of hyperunstable
α Hb variant. It is a rare cause for α-thalassaemia-intermedia
when the mutation combines with a αα deletion defect. If the
mutation occurs in the αα-globin gene it results in hydrolys
fetalis. mRNA formation in αααα1 is 3:1 and accounts for the
condition being more severe when the αα gene is involved.
The two cases first reported with α-thalassaemia-intermedia
were from Turkey. The true frequency of the molecular
defect in the α-globin 1 gene CD59 (GGC-GAC) is unknown
as the defect can only be ascertained by DNA studies. This
α Hb variant is hyperunstable and has no product to be
visualized by routine haematology studies. Screening for
thalassaemia by blood counts and Hb subtyping will miss the
diagnosis in contrast to beta-thalassaemia where
determination of Hb subtypes presumptively identifies the
presence of thalassaemia.

This is the first report described in a Malay patient from
Malaysia. The presence of ααCD59 (GGC-GAC) may be the
result of migration from countries bordering the
Mediterranean sea as the Malays have links with Turkey, Iran
and the Middle East. The true frequency of this alpha Hb
variant cannot be identified by screening methods such as the
BHER protocol as it is hyper-unstable. This case report
highlights the need to consider a hypersunstable α Hb variant
[CD59 (GGC-GAC)] in a patient with alpha thalassaemia-
intermedia in the absence of a strongly positive H-inclusion
test with normal Hb subtypes and presence of Hb Barts.
Confirmation is required by DNA analysis as shown in this
case.

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