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CASE REPORT

Family study of haemoglobin Arya in a Malaysian family

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Abstract

Introduction: Thalassemia and haemoglobinopathies are relatively common among Malaysians. One of the rare haemoglobinopathies reported is Haemoglobin (Hb) Arya, which occurs due to substitution of aspartic acid at residue 47 of the alpha chain by asparagine. Here, we report the detection of Hb Arya in a Malaysian family, which was detected incidentally during family screening. *Case Report:* A 16 years-old girl, clinically asymptomatic was noted to have low mean corpuscular haemoglobin (MCV) with normal Hb level. Hb analysis using capillary electrophoresis (CE) showed reduced Hb A of 76.5%, Hb A2 of 1.6% with presence of small peak at Zone 1 likely A2'. There was also a small peak noted at Hb D zone and Hb S zones which quantified as 1.5% and 20% respectively. Supplementary test by high performance liquid chromatography (HPLC) showed a prominent peak at D-window (19.6%) and a small peak at S-window (0.6%). DNA analysis revealed a heterozygous state of α2 codon 47 Hb Arya mutation. Subsequent family study showed a similar mutation in the father and sister of the index case. *Conclusion:* Very few reports are available up to date regarding Hb Arya. This report highlights the rare haemoglobinopathy in a Malay family in Malaysia that contributes to the growing literature of this rare haemoglobin variant.

Keywords: Haemoglobin Arya, Haemoglobin variant

INTRODUCTION

Thalassemia is a common disorder in Malaysia. Southeast Asean countries have a higher prevalence of α thalassemia, with the prevalence in Malaysia being 4.1%.[1] The thalassemia screening program among secondary school students has proven to identify carriers, and make informed decisions about having children, and consequently decreased the number of thalassemia major cases.[2]

The Malaysian thalassemia screening program mainly takes into account the mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) as significant predictive factors in diagnosing thalassemia carriers.[2] In a study done by Institute of Medical Research (IMR) in 2013, it was observed that the mean MCV for silent α thalassemia carriers were 75.8 fL and that of both, α^+ and α^0 thalassemia traits were consistently lesser than 71 fL. While, MCH was 24.2 pg in silent carriers and 21.8 pg in α^+ and α^0 thalassemia trait individuals.[1] These two parameters are thus able to provide a rough guide in differentiating silent α thalassemia carriers from α^+ and α^0 thalassemia traits.[1] Besides that, screening for thalassemia / haemoglobinopathies also enables the detection of many rare Hb variants. One of the extremely rare Hb variants detected thus far is Hb Arya.

Hb Arya (HBA2:c.142G>A) was first described in an Iranian family in 1974.[3] In this Hb variant, the substitution occurs at residue 47 GAC>AAC [Asp>Asn] of the α chain where aspartic acid has been substituted by asparagine. Due to the gene conversion, Hb Arya has a very close electrophoretic mobility to that of Hb S. This mimics an electrophoretic pattern of Hb S carrier.[4] Clinically, patients with Hb Arya are asymptomatic and they are only detected incidentally. No clinical manifestations have been reported so far.

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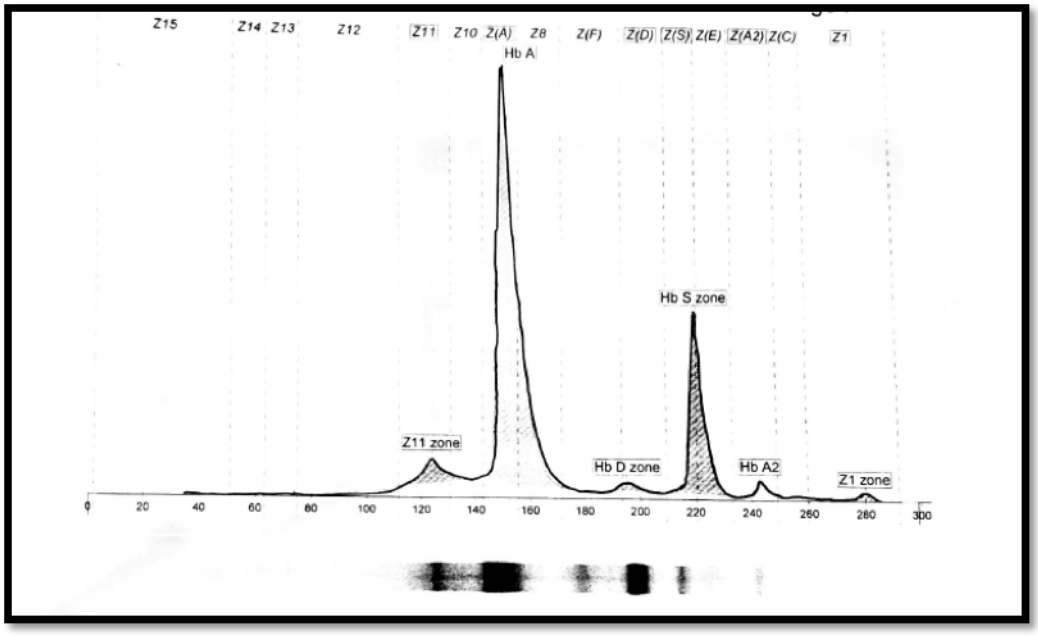
CASE REPORT

The index case was a 16-year-old Malay girl who was screened for thalassemia / haemoglobinopathy under the Malaysian thalassemia screening program for form four students. She has no past medical or surgical histories and no significant family history. She was the second child among three children in a non-consanguineous marriage. Clinically, she has no hepatosplenomegaly and no other significant abnormalities were detected.

The index case showed normal Hb levels with raised red blood cell (RBC) of $5.14x10^{12}$/L, low MVC and MCH of 78.4 fl and 26.3 pg respectively. Peripheral blood smear showed hypochromic microcytic red cells with no other significant red cell abnormalities. Her Hb analysis by capillary electrophoresis (CE) showed relatively low Hb A levels (76.5%) and low Hb A_2 levels (1.6%) with a small peak noted at Hb D zone (1.5%) and a prominent peak at Hb S zone (20%). A small peak of 4.4% was seen at Zone 11, likely due to denatured Hb A. A small peak at Zone 1, likely to be A2' was also present. The second method by high performance liquid chromatography (HPLC) showed a prominent peak at retention time (RT) 4.18 of 19.6% and 0.6% at RT 4.57 min, which is likely A' peak which indicates an alpha variant (Figure 1). The sickling test was negative. DNA analysis using Sanger sequencing showed the presence of a heterozygous state of α2 codon 47 (GAC>AAC) Hb Arya mutation (Figure 2).

Following these findings, family study for thalassemia / haemoglobinopathies was conducted. Her father and sister had normal Hb, MCH and MCV values, while her mother and brother had normal Hb levels and low MCV and MCH values. All the family members had RBC counts ranging from 5.01 - $5.91x10^{12}$/L. Other full blood count (FBC) parameters were all within normal range. The findings of peripheral blood smear of her mother and brother showed hypochromic microcytic red cells, similar to the



Name	%
Z11 zone	4.4
Hb A	72.1
Hb D zone	1.5
Hb S zone	20.0
Hb A2	1.6
Z1 zone	0.4

Figure 1A: Capillary electrophoresis of the index case.

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
P1	---	0.1	0.76	1389
Unknown	---	0.1	1.00	1364
F	0.3	---	1.10	5790
Unknown	---	0.6	1.25	13127
P2	---	2.9	1.35	67249
P3	---	8.7	1.72	198859
Ao	---	64.0	2.38	1460100
A2	3.4*	---	3.61	73100
D-window	---	19.6	4.18	447818
S-window	---	0.6	4.57	12713

Total Area: 2,281,512

F Concentration = 0.3 %
A2 Concentration = 3.4* %

*Values outside of expected ranges

Analysis comments:

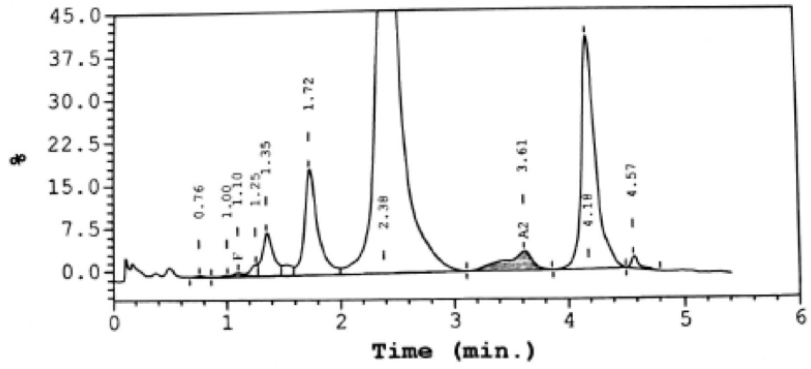


Figure 1B: HPLC of the index case.

index case. However, her father's and sister's blood smears were unremarkable. The CE findings of her father and sister showed relatively low Hb A and Hb A2 levels and both have a prominent peak at Hb S zone, 21.7% and 21.8% respectively. The subsequent HPLC analysis of her father and sister showed a prominent peak at RT 4.17 (19.8%) and RT 4.19 (20.3%) respectively. Sickling test was negative in her father and sister. Her mother and brother had normal CE findings and HPLC was not done. Similar to the index case, molecular analysis for α gene clusters confirmed the presence of a heterozygous state of α2 codon 47 (GAC>AAC)

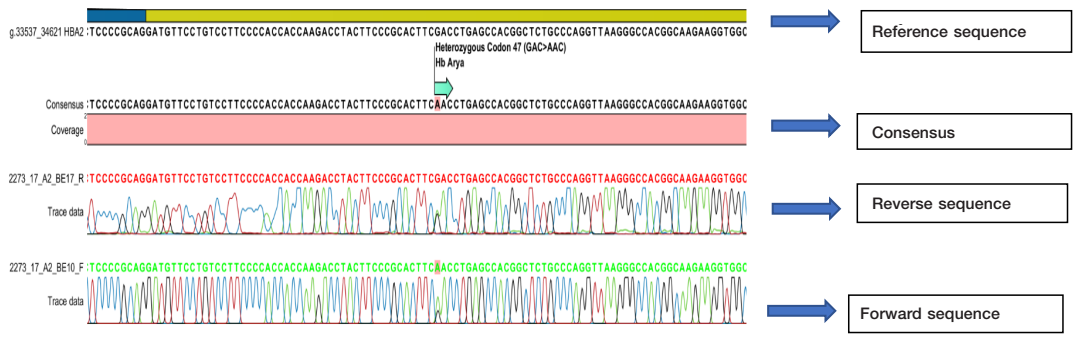


Figure 2: Sanger sequencing showing presence of α2 codon 47 (GAC>AAC) Hb Arya mutation in index.

Hb Arya mutation in her father and sister. While, her mother and brother were noted to have a heterozygous α plus thalassemia (-$\alpha^{4.2}$) deletion, which was not present in the index case, her father and sister. The index case and her family members were not further followed up by the clinician as they were all asymptomatic. Summary of FBC, FBP findings, Hb analysis and DNA analysis of index case and her family members are represented in Table 1 below.

DISCUSSION

Haemoglobinopathies are a heterogenous group of diseases caused by disruption in the normal expression of genes encoding the globin chains.[5] Mutation of the globin gene affecting Hb is common worldwide, affecting approximately 7% of the world's population.[6] These mutations are subdivided into those that impairs the globin protein subunit production, leading to thalassemia; and those that produce a structurally abnormal globin protein, leading to Hb variants.[6]

Hb variants result from substitutions of amino acids in the alpha, beta, gamma or delta chain tetramers of Hb A, F and A2. Changes in the DNA nucleotides, such as deletions, insertions or point mutations in one of these globin genes, form Hb variants.[5]

Hb variants can be classified into several groups, such as those with unstable mutants (Hb Philly, Hb Prato); high affinity variants (Hb Hiroshima); low affinity variants (Hb Kansas); methaemoglobin variants (Hb M-Saskatoon); globin chain elongation variant (Hb Constant Spring); and variants with multiple effects (Hb E).[6] Many of these Hb variants are clinically silent, however some Hb variants produce clinical manifestations of varying severity.[5]

Hb variants are usually detected by their changes in physiochemical properties occurring from the amino acid modifications. The two most exclusively used methods used in our routine practice are HPLC and CE.[7,8,9] These method

Table 1: FBC, FBP, Hb analysis and DNA analysis findings all index case and her family

Laboratory investigations	(Index case)	Father	Mother	Sister	Brother
FBC					
RBC (x10^{12}/L)	5.14	5.7	5.61	5.01	5.91
Hb (g/L)	135	158	134	140	139
MCV (fL)	78.4	83	75	85.5	72.9
MCH (pg)	26.3	27.7	23.9	27.9	23.6
FBP (significant findings)	Hypochromic microcytic red cells	Normo-chromic normocytic red cells	Hypochromic microcytic red cells	Normochromic normocytic red cells	Hypochromic microcytic red cells, few pencil cells
CE					
HbA (%)	76.5	76	97.3	75.9	97.3
HbA2 (%)	1.6	1.7	2.7	1.8	2.7
Other window	HbD 1.5% HbS 20%	HbS 21.7%	NA	HbS 21.8%	NA
HPLC					
HbA (%)	64	68.4	NA	68.4	NA
HbA2 (%)	3.4	1.8	NA	1.9	NA
HbF (%)	0.3	0.1	NA	0.3	NA
Other peaks:					
RT4.17-4.19	19.6%	19.8%	NA	20.3%	NA
RT 4.56-4.58	0.6%	0.6%	NA	0.6%	NA
DNA analysis	Hb Arya	Hb Arya	Heterozygous alpha plus 4.2 deletion	Hb Arya	Heterozy-gous alpha plus 4.2 deletion

can lead to a putative identification of a variant, however, it should be interpreted cautiously as a similar mobility can be produced by several amino acid changes.[4] In many cases, it is impossible to identify a variant only by using CE and HPLC, as some variants mimicking Hb S are regularly reported, as in this family study.[7,8,9]

Based on the evidence of the limited number of published cases on Hb Arya in online journal search, it shows evidence that this Hb variant is very rare. It was first described in an Iranian lady back in 1974.[3] The case reported in this case report is the first case reported in Malaysia. This haemoglobinopathy is detected upon further investigation of abnormal findings on Hb analysis assessment and confirmed by DNA analysis.

Hb Arya occurs due to substitution of aspartic acid to asparagine at α2 Codon 47. Mutations occurring at the residue 47 is not known to affect the dissociation of oxygen and does not cause Hb instability.[3] Hence, this haemoglobinopathy is reported to be clinically silent. Other Hb variants with similar properties are Hb Hasharon which occurs due to substitution of histidine and Hb L-Ferrara where glycine substitution occurs.[3] In a case report by Charache et al in 1969, it was suggested that Hb Hasharon is unstable in vivo and mild haemolytic anaemia can occur.[10]

In this family study, the index case, her father and sister were confirmed to have Hb Arya. They were detected incidentally. However, the mother and brother of the index case were noted to have heterozygous α plus thalassaemia ($-\alpha^{4.2}$) deletion.

Almost all cases of Hb Arya have normal Hb levels with normal MCV and MCH.[3] In the cases reported here, only the index case had low MCV and low MCH; however, the father and sister had normal MCV and MCH. In regards to this low MCV and MCH in the index case, iron deficiency was not excluded and the index case did not have α(4.2) gene deletion.

Certain Hb variants can have an electrophoretic mobility similar to that of Hb S. This is seen in Hb Arya and Hb Hasharon.[4,10] Usually, the prominent peak at Hb S zone is more than 20%.[4] This accurately mimics a state of Hb S carrier. In order to rule out Hb S, sickling test should be performed. In this case report, the sickling test results were negative for all the 3 cases with Hb Arya.

It is important to be aware that HPLC and CE are the most cost-appropriate measure for screening of thalassemia / hemolobinopathies. However, for a proper diagnosis to be made, additional methods such as DNA analysis is utmost important to not miss other diagnosis that could mimic some pathogenic ones, such as Hb S carrier, as observed in this case.[4]

CONCLUSION

Among all the haemoglobinopathies, Hb Arya is a very rare haemoglobinopathy. Indeed, given the diversity of Hb variants found in our population, we highlighted this rare disease in Malaysia contributes to the growing literature of the disorder. Besides that, it is also utmost important to be aware of this Hb variant to avoid misdiagnosis of Hb S.

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