International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Case Report

A Rare Case of Compound Heterozygous Haemoglobin Q-Thailand and Haemoglobin Adana

Ezalia Esa[1], Tan Jen Ern[2], Rahimah Ahmad[1], Nur Aisyah Aziz[1], Zubaidah Zakaria[1], Azlinda Abu Bakar[3]

[1]Haematology Unit, Cancer Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia
[2]Paediatric Department, Hospital Melaka, Jalan Mufti Haji Khalil, 75400 Melaka, Malaysia
[3]Pathology Department, Hospital Melaka, Jalan Mufti Haji Khalil, 75400 Melaka, Malaysia

Corresponding Author: Ezalia Esa

Received: 23/07//2014 *Revised: 09/08/2014* *Accepted: 14/08/2014*

ABSTRACT

We report a 15-year-old Malay girl who since the age of five required infrequent blood transfusions. However over the past 10 years, her transfusion requirements increased due to symptomatic anaemia. Currently, she has become transfusion dependent. Her peripheral blood film was consistent with severe haemolytic anaemia and the H-inclusion test was negative. Her mother, who was 36-week pregnant, also had features of haemolytic anaemia (haemoglobin, Hb: 8.5 g/dl) but in a milder form. Her father had normal Hb level but raised red cell count. Both parents had negative H-inclusion test. Hb subtyping by cation exchange high performance liquid chromatography and capillary electrophoresis only revealed presence of Hb variant in the father and the daughter. The abnormal peak appeared at retention time 4.62 seconds (25.6%) and zone 7 (28.2%) in the father. Similar findings were observed in the daughter, but with lesser values (retention time 4.58 seconds, 15.1% and zone 7, 19.1%). Multiplex Gap PCR revealed that both of them were heterozygous for single α-globin gene deletion ($\alpha^{-4.2}$). Further analysis of α_1- and α_2-globin gene by DNA sequencing had confirmed they were heterozygous for Hb Q-Thailand, and surprisingly, the daughter were also found to have heterozygous Hb Adana in α_2-globin gene. The inheritance of Hb Adana was then discovered when the mother was found to be heterozygous for α_2 Hb Adana by Mutiplex ARMS PCR. Compound heterozygosity of Hb Q-Thailand and Hb Adana, which is a highly unstable Hb variant, would explain the clinical phenotype that was manifested by the patient.

Keywords: alpha thalassemia intermedia, Hb Q-Thailand, Hb Adana, hyperunstable Hb variant.

INTRODUCTION

Alpha thalassaemia can either result from deletions or point mutations of α-globin gene, in which deletions is the commoner mutation. The deletion defects can be due to loss of one α-globin gene (α^+), both α-globin genes in tandem (α^0), and the entire ζ-α-globin gene cluster. These deletional types lead to deficient or absent synthesis of α-globin chain. Meanwhile, the non-deletion defects (point mutations) would generally produce the α-globin chain variants of no clinical significance. However, if the mutations involve critically positioned amino-acid residues, they might

generate the highly unstable haemoglobins that can give rise to haemolytic anaemia. [1]

Haemoglobin (Hb) Q-Thailand is an α-globin chain variant that is caused by a point mutation in codon 74 of the α_1-globin gene on chromosome 16p (GAC→CAC) resulting in substitution of aspartic acid to histidine (Asp→His). As the mutation is invariably associated with a leftward single α-globin gene deletion ($-\alpha^{4.2}$), individuals with heterozygous Hb Q-Thailand usually show slight red cell microcytosis. The carriers are always asymptomatic since the Hb variant is stable and has normal oxygen affinity. [2] On the other hand, Hb Adana, is one of the highly unstable α-globin chain variants. It arises from point mutation of codon 59 in α_1- or α_2-globin genes (GGC→GAC) resulting in replacement of glycine to aspartic acid (Gly→Asp). Most of the highly unstable α-globin variants are identified when they interact with other alpha thalasaemia, typically causing Hb H disease, or occasionally, a state similar to beta thalassemia intermedia. [3,4] In Hb Adana, it is not only such interactions that would influence the clinical phenotype, but also the type of α-globin gene that is mutated (either α_1- or α_2-globin gene) and the degree of protein instability in polypeptide globin chain. [1,3,4] To the best of our knowledge, this is the first case of alpha thalassaemia intermedia in Malaysia which shows the interaction of heterozygous Hb Q-Thailand with the rare α_2 Hb Adana.

CASE REPORT

The case is a 15-year-old Malay girl who was known to receive occasional blood transfusions since she was five years old. Her condition had progressively worsened over the past ten years, and in July 2012 she became transfusion dependent. On examination, she appeared pale. The liver and spleen were palpable, three finger breaths below the respective costal margin. Her 49-year-old father was asymptomatic and had no known history of thalassaemia. However, her 37-year-old mother who was 36-week pregnant had always experienced anaemic symptoms during pregnancy. She was also pale, but otherwise no obvious abnormality was detected in other systems.

Table 1. Phenotype and genotype findings of the patient and her parents.

Parameter	Normal Range	Father	Mother (pregnant)	Daughter (patient)
RBC ($x10^{12}$/L)	3.80-4.80	5.48	2.90	2.95
Hb (g/dL)	12.0-15.0	14.5	8.5	7.4
MCV (fL)	83.0-101.0	83.0	93.0	82.0
MCH (pg)	27.0-32.0	26.5	29.5	24.9
RDW (%)	11.6-14.0	14.3	25.2	20.0
α-globin genotype	αα/αα	($\alpha\alpha^{Adana}/-\alpha^{4.2\text{-}QT}$)	($\alpha\alpha^{Adana}/\alpha\alpha$)	($\alpha\alpha^{Adana}/-\alpha^{4.2\text{-}QT}$)

RBC: Red blood cell, Hb: Haemoglobin, MCV: Mean cell volume, MCH: Mean cell haemoglobin, RDW: Red blood cell distribution width

The haemogram from an automated blood counter (Beckman Coulter DXH 800, Beckman Coulter Inc., USA) showed that the patient and her mother had anaemia, 7.4 g/dl and 8.5 g/dl respectively (Table 1). The peripheral blood film of the patient was consistent with severe haemolytic anaemia (Figure 1), while in the mother a milder form of haemolytic features were seen. Blood count of her father showed typical features of thalassaemia trait with normal Hb level (14.5 g/dl), high red cell count and hypochromia. All of them had negative H-inclusion test. High performance liquid chromatography (HPLC) using Biorad Variant Haemoglobin Testing System (Hercules, USA) revealed presence of abnormal peak in the patient and her father

(Figure 2a). It appeared at retention time of 4.58-4.62 seconds with percentage of 19.1% (patient) and 25.6% (father). Using Sebia Capillarys (Sebia, France), the Hb variant was also detected by capillary electrophoresis method as it appeared in zone 7 (patient, 19.1% and father, 28.2%) (Figure 2b). Hb electrophoresis using automated agarose gel, Interlab G26 (Interlab, Italy) on alkaline pH (8.6) clearly showed Hb variant that migrated anodal to Hb A. No Hb variant was detected in the mother using these three screening methods.

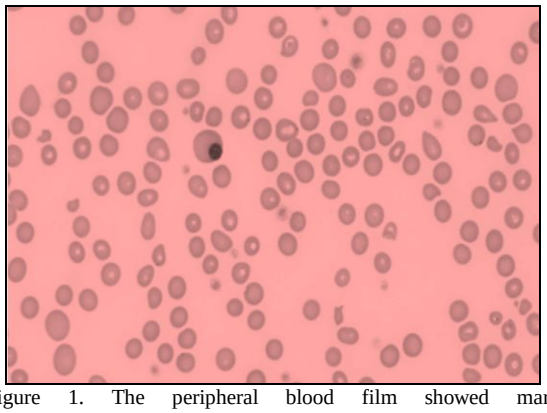

Figure 1. The peripheral blood film showed mark anisopoikilocytosis and presence of target cells, tear drop cells and few fragmented RBCs. There was increase erythropoiesis as shown by raised polychromatic cells and presence of a few circulating nucleated RBCs.

(a) (b)

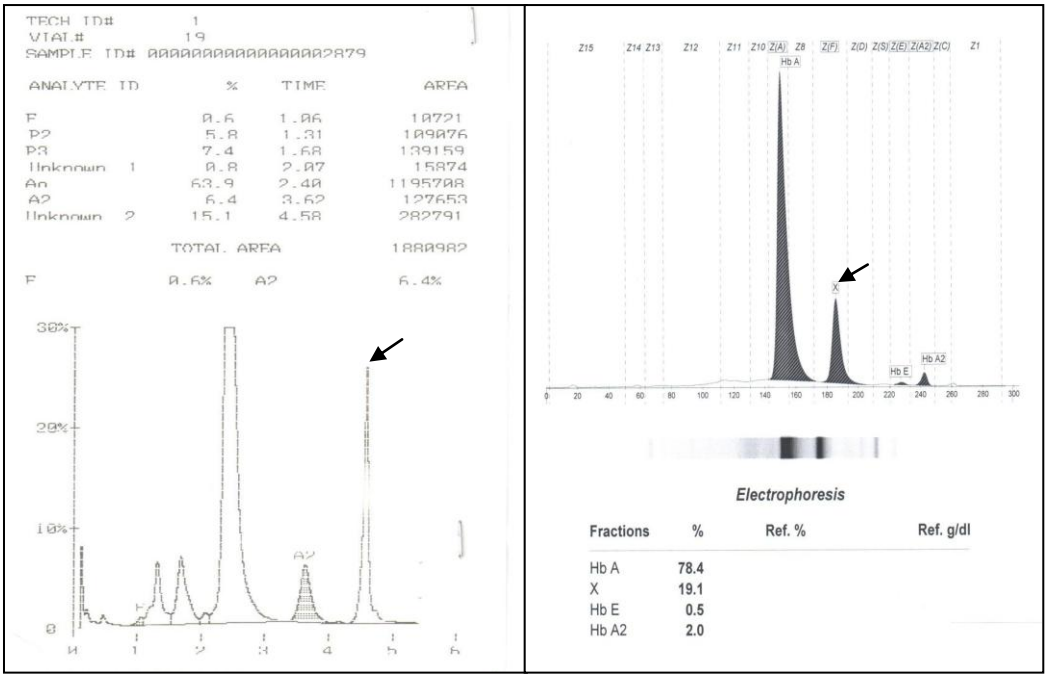

Figure 2. Hb subtyping of the patient: (a) HPLC showed abnormal peak (arrow) at retention time 4.58 seconds (15.1%). (b) Capillary electrophoresis showed Hb variant (arrow) at zone 7 (19.1%).

DNA studies were performed on extracted DNA. Multiplex GAP-PCR capable of detecting single ($-\alpha^{3.7}$, $-\alpha^{4.2}$) and double α-globin gene deletion ($--^{SEA}$, $--^{FIL}$, $--^{MED}$, $--\alpha^{20.5}$ and $-^{THAI}$) showed the presence of a heterozygous single gene deletion ($-\alpha^{4.2}$) in both the father and index patient (Figure 3). DNA sequencing analysis of the amplified α_1- and α_2-globin gene of both father and index confirmed that they had point mutation (GAC→CAC; Asp→His) at codon 74 of the α_1-globin gene, indicating that they were carriers of Hb Q-Thailand (Figure 4). In addition the daughter also inherited another point mutation in α_2-globin gene, the codon 59 mutation (GGC→GAC;

Gly→Asp) Hb Adana (Figure 5). This heterozygous inheritance of Hb Adana was also discovered by Mutiplex ARMS PCR which is able to detect the α-globin gene point mutations [initiation codon (ATG→GAC), codon 30 (ΔGAG), codon 35 (TCC→CCC), codon 125 (CTG→CCG), termination codon (TAA→CAA) and the codon 59 mutation (GGC→GAC]. This analysis revealed that the mother was a carrier for α_2 Hb Adana.

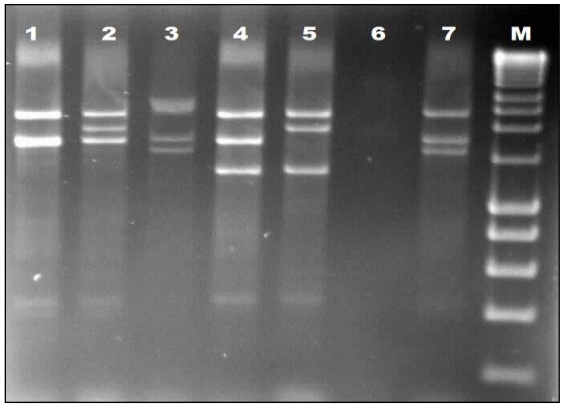

Figure 3. Multiplex GAP-PCR showed lane 1: α_2 control, lane 2: -$\alpha^{3.7}$ control, lane 3: -$\alpha^{4.2}$ control, lane 4: --^{SEA} control, lane 5: -$\alpha^{3.7}$/--^{SEA} control, lane 6: blank, lane 7: DNA from the patient and lane M: hyperladder I. From this analysis, the patient was heterozygous single gene deletion (-$\alpha^{4.2}$).

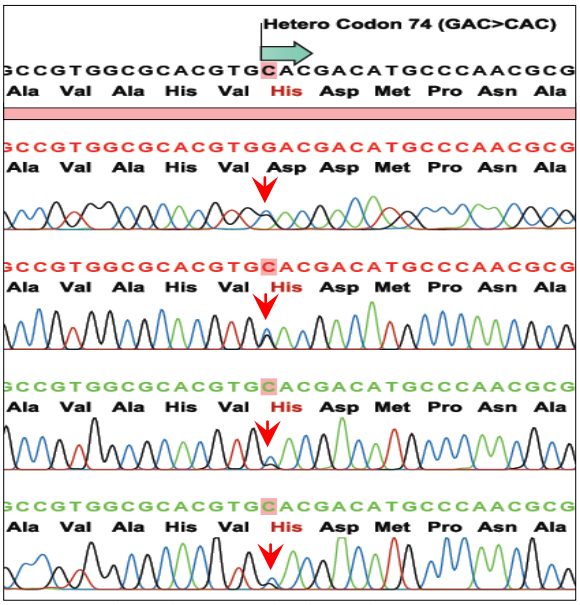

Figure 4. Electropherogram of codon 74 in α_1-globin gene showed a point mutation G>C (GAC→CAC; Asp→His; HBA1:c.223G>C) in heterozygous state (red arrow).

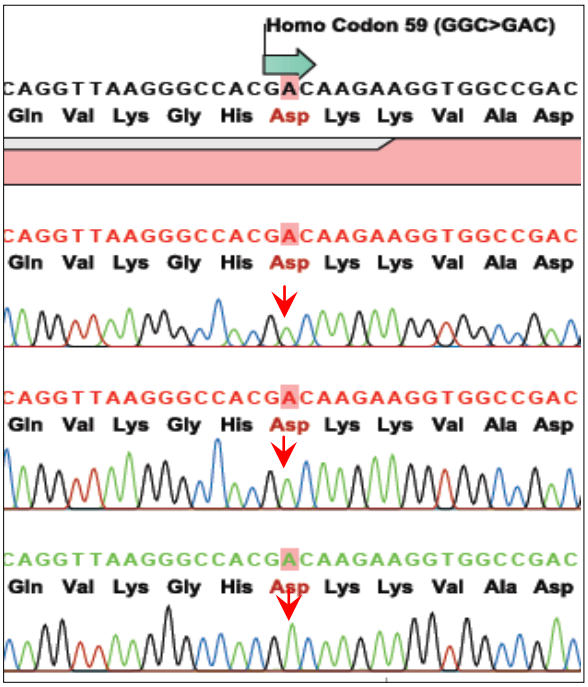

Figure 5. Electropherogram of codon 59 in α_2-globin gene showed a point mutation G>A (GGC→GAC; Gly→Asp; HGVS, HBA2:c.179G>A) in heterozygous state (red arrow). In this figure, the mutation was depicted in homozygous state due to $\alpha^{-4.2}$ deletion.

DISCUSSION

Patients with alpha thalassaemia intermedia usually appear normal at birth but often develop anaemia and splenomegaly by the end of the first year. Their Hb levels are around 7-10 g/dl and often require infrequent blood transfusion. However, some patients might progress into severe state especially among those with non-deletional mutations.

HB H disease is the commonest cause of alpha thalassaemia intermedia. Commonly Hb H disease results from three α-globin gene deletion (--/-α). Patients with deletional Hb H disease rarely require blood transfusion except during acute haemolytic crisis induced by infections, pregnancy and surgery. Alternatively, Hb H disease can also result from the interaction between α^0 deletion and non-deletional α thalassaemia (--/$\alpha^T\alpha$ or --/$\alpha\alpha^T$) in which Hb H-Constant

Spring (--/$\alpha^{CS}\alpha$) is the most common form of non-deletional Hb H disease. [5,6]

HB Constant Spring is able to be presumptively identified by most of the current screening methods and therefore would prompt for further DNA study. Hb Constant Spring is a termination codon mutation defect (TAA→CAA) that leads to a 31-residue extension of the expressed α-globin chain variant. The clinical manifestation of Hb H-Constant Spring is more severe than deletional Hb H disease because the former is related to unstability of the variant that causes red cell membrane damage. [6] The phenotype is more evident when the non-deletion defect interacts with another non-deletional α-gene mutation ($\alpha^T\alpha/\alpha^T\alpha$ or $\alpha^T\alpha/\alpha\alpha^T$) e.g. Hb Adana. Hb Adana is relatively rare in Malaysia (0.14 to 3.7%) but quite common in Indonesia (16%). [6-8] Recently nine cases of double heterozygous for non-deletional alpha thalassaemia in trans to α_2 Hb Adana were reported among the Indonesian population. The findings in this Indonesian study shows that the heterogeneity of the clinical course is dependent on both genetic and non-genetic modifiers. [7]

Our case was compound heterozygous for Hb Adana in α_2 gene and Hb Q-Thailand ($\alpha^{Adana}\alpha/-\alpha^{4.2\text{-}QT}$). The diagnosis work out was quite challenging since Hb Q-Thailand was the only possible diagnosis after testing using the routine screening assays. Since Hb Adana is hyperunstable, it is thus not able to be visualised by the routine screening methods.[5] In fact the presence of Hb Adana was able to be identified by DNA analysis of the α_2 globin gene. The diagnosis of co-inheritance of heterozygous Hb Adana would have been easily missed in this patient if the clinical history was not taken into account. It is known that carriers for Hb Q-Thailand are asymptomatic as seen in the father. [2] It was very unlikely that the patient was only heterozygous Hb Q-Thailand since she was transfusion dependent. Therefore, it is always essential to consider co-inheritance of hyperunstable alpha Hb variant in a patient with alpha thalassaemia intermedia. Definitive diagnoses by DNA analysis including parental studies are required as shown in this case.

ACKNOWLEDGEMENT

We would like to express our appreciation to Director General of Health, Malaysia for his permission to publish and the Deputy Director General of Health (Research and Technical Support, MOH) for her continuous support. We would like to extend our appreciation to the Director, Institute for Medical Research and Head of Cancer Research Centre for their encouragement. Lastly our appreciation to Hospital Kuala Lumpur who has kindly performed Capillary Electrophoresis for our sample.

REFERENCES

1. John SW, David HKC. The α-globin Gene Cluster: Genetics And Disorders. Clin Invest Med 2001 Apr;24(2):103-9.
2. Singsanan S, Karnpean R, Fucharoen G, Sanchaisuriya K, et al. Hemoglobin Q-Thailand Related Disorders: Origin, Molecular, Hematological And Diagnostic Aspects. Blood Cells Mol. 2010 Dis;45: 210-14.
3. Traeger-Synodinos J, Metaxotou-Mavrommati A, Karagiorga M, et al. Interaction Of An α+-Thalassaemia Deletion With Either a Highly Unstable α-Globin Variant (α2, Codon 59, GGC→GAC) Or A Non-Deletional α-Thalssaemia Mutation (AATAAA→AATAAG): Comparison of Phenotypes Illustrating "Dominant" α-Thalassaemia. Haemoglobin 1999.23(4):325-37.
4. Fucharoen S, Viprakasit V. Haemoglobin H Disease: Clinical Course and Disease Modifiers. Hematology American Society of

Hematology Education Programme. 2009;26-34.
5. George E, JAMA Tan, AS Nor Azian, et al. A Rare Case Of Alpha-Thalassaemia Intermedia In A Malay Patient Double Heterozygous For α+-Thalassaemia And A Mutation In α1 Globin Gene CD59 (GGC→GAC). Case Report. Med J Malaysia. 2009 Dec;64(4):321-22.
6. Wajcman H, Traeger-Synodinos J, Papassotiriou I, et al. Unstable And Thalassemic α Chain Hemoglobin Variants: A Cause of Haemoglobin H Disease And Thalassaemia Intermedia. Hemoglobin 2008;32(4): 327-49.
7. Nainggolan IM, Harahap A, Ambarwati DD, et al. Interaction Of Haemoglobin Adana (Haemoglobin A_2: c.179G>A) With Deletional And Nondeletional α(+)-Thalassemia Mutations: Diverse Hematological And Clinical Features. Hemoglobin. 2013;37(3):297-305.
8. Rahimah A, Saleem M, Nisha Sabrina A, et al. Distribution Of Alpha Thalassaemia Gene Variants In Diverse Ethnic Populations In Malaysia: Data From The Institute For Medical Research. International Journal of Molecular Sciences. 2013;14:18599-614.

How to cite this article: Esa E, Ern TJ, Ahmad R et. al. A rare case of compound heterozygous haemoglobin Q-Thailand and haemoglobin Adana. Int J Health Sci Res. 2014;4(10):327-332.

****