ISSN: 2394-0026 (P)
ISSN: 2394-0034 (O)

Case Series

Hb Q – India: An uncommon hemoglobin variant diagnosed in two patients – Case series

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How to cite this article: Rippalkumar Bhimani, Gunvanti B. Rathod, Sachin Aggarwal, Rushabh Patel, Rahul Goyal, N.K. Kuchhal. Hb Q – India: An uncommon hemoglobin variant diagnosed in two patients – Case series. IAIM, 2014; 1(4): 68-74.

Available online at www.iaimjournal.com

Received on: 14-11-2014 **Accepted on:** 10-12-2014

Abstract

Hemoglobin Q-India (α 64 Asp→His) is an important member of the hemoglobin Q family, molecularly characterized by the replacement of aspartic acid by histidine. The first case of Hb Q-India was reported by Sukumaran in 1972 in a Sindhi family with associated β-Thalassemia. India is known as a country with a high prevalence of α - and β-thalassemia and different types of hemoglobinopathy. Many of these variants are yet to be identified. Here, we are reporting two cases of Hb Q- India diagnosed during premarital thalassemia screening.

Key words

Hemoglobin Q-India, Hemoglobinopathy, Aspartic acid, Histidine.

Introduction

Hemoglobin has plenty of variants [1]. Hemoglobin (Hb) Q is a single nucleotide polymorphism occurring in the Hb α -2 chain. Hb Q variants are recognized by a slow-moving band migrating at a similar position to Hb S on alkaline pH electrophoresis. This Hb Q variant has normal solubility [2]. A number of important members of the Hb Q family share a certain molecular feature - the replacement of aspartic acid (Asp) by histidine (His) at different positions in the amino acid chain. These include Hb Q-Thailand (α 74 Asp→His), Q-India (α 64

ISSN: 2394-0026 (P)
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Asp→His) and Q-Iran (α 75 Asp→His). Hemoglobin Q is a rare alpha chain variant first described by Vella et al. [3] in association with α -Thalassemia in a Chinese family. The first case of Hb Q-India was reported by Sukumaran [4] in 1972 in a Sindhi family with associated β-Thalassemia and later by Desai [5]. Hb Q India is not having deleterious phenotypic effect [4]. Hereby, we report two cases which includes 21 years old female and 27 years old male who are carriers of Hb Q India gene diagnosed at our laboratory.

Case reports

A 21 years old female and 27 years old male from Delhi, India came to laboratory for investigation of premarital thalassemia screening. Hemograms were performed on fully automated KX-21 analyser. The male patient showed no anemia or reticulocytosis and MCV, MCH were normal. While the female patient showed anemia and normal MCV but normal to slightly reduced MCH as per **Table – 1**. On Bio-Rad cation exchange HPLC, a unknown peak was seen in the P3 in the retention time of 3.78 minute and 3.79 mintue respectively for female and male patient. **(Photo – 1, Photo – 2)** Here, we found the amount of Hb Q-India to be 14.6% and 15.8% respectively in female and male patient as per **Table – 2** and **Table – 3**.

Discussion

Hb Q-India is a rare α chain structural variant caused by a mutation in the position of codon 64 of α - 1 gene with a change of Asp→ His. The prevalence of Hb Q- India in India is 0.4%, found predominantly in Sindhi families and in individuals from western and northern India. Hb Q-India levels in heterozygotes are normally below 20% and reduce further in interactions with β thalassemia [6].

Computerized simulation of secondary and tertiary protein structures of these Hb molecules by standard bio-informatic methods suggest that Hb Q-India has a protein structure similar to the normal Hb molecule [7]. In the heterozygous state, patients with Hb Q-India or Hb Q-Iran do not have the thalassemia phenotype or any distinctive clinical manifestation. Furthermore, these Hb abnormalities do not affect hematologic features. The replacement of aspartic acid with histidine is on the surface of the protein structure and does not affect the protein inter chain contacts and electrical charges of the molecule, and therefore does not cause any changes in hematologic parameters and indices [8]. Interestingly, our female patient with Hb Q-India suffered from mild anemia. Normally Hb Q is clinically silent. Even its presence along with beta thalassemia trait does not seem to produce any clinical abnormality. But, HbQ-H disease can give clinical manifestations though it is very rare [9]. Quantities of HbQ variant is usually determined by the ratio of alpha A, alpha Q and beta A globin chains. Presence of alpha thalassemia favors the formation of HbQ, whereas beta thalassemia reduces the formation of HbQ [10, 11].

The molecular characterization of hemoglobin variants is usually conducted at two levels. The first level involves gel electrophoresis or cation exchange high performance liquid chromatography (HPLC) while the second level of analysis engages Mass Spectrometry (MS) [12, 13] and/or DNA sequencing [12]. A small number of Hb variants can be characterised by comparing their HPLC retention times with reference chromatograms in a library provided by the manufacturer, and also by comparing their IEF positions with those on a published chart of abnormal Hb variants [14].

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In general, HPLC and IEF provide reliable and reproducible data, enabling the retention time and IEF position to be used to identify variants indirectly. However, several variants are known to have identical retention times and the same applies to IEF positions. Thus, the data are limited in suggesting a candidate variant when only one technique is used. A more accurate identification is obtained when both HPLC and IEF are performed and the HPLC retention time and the IEF position match the data of a known variant. The combination of liquid chromatography and Electrospray ionization Mass Spectrometry (LC-ESI-MS) [15] and DNA sequencing analysis are complementary techniques, with the latter usually being used to confirm deductions based upon mass spectrometric analysis.

One of the important methods for the detection of the various abnormal haemoglobins is ARMS-PCR. This is quite useful for the quick identification of the α chain variant Hb Q-India and to identify any uncommon variant of the α globin or β globin genes for which the mutation is known. For the many variants that have not had their causative point mutation confirmed by DNA sequence analysis, an ARMS primer would have to be designed to detect the presumed mutation predicted by the genetic code and the amino acid change. However, all new Hb variants are now characterised by DNA sequence analysis, and the number of variants having their mutation confirmed by DNA sequencing is growing, enabling a panel of ARMS primers to be developed that would be specific to the local spectrum of Hb variants.

The definitive method for the identification of Hb variants is characterisation by DNA sequencing of the α globin and β globin genes. This is an expensive technique and not practical for the routine identification of uncommon variants. Because most α chain and β chain variants result from a single point mutation, a simple, rapid, and inexpensive method of diagnosing point mutations is required for the definitive characterisation of the uncommon haemoglobin variants.

Conclusion

India is known as a country with a high prevalence of different types of hemoglobinopathy. Many of the Hb variants are yet to be identified. Nowadays, HPLC, IEF, ARMS-PCR, DNA sequencing are the methods available for the diagnosis of the abnormal Hb like Hb Q-India. We stress on the point that careful screening of the samples using routine techniques like Hb electrophoresis and chromatography can be the basis of identification of abnormal haemoglobin variants.

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Table – 1: Parameters of blood count.

	Age	Hb (g/l)	RBC (×10 12/l)	MCV (fl)	MCH (pg)	MCHC (%)
Case 1	21 years	10.2	4.01	84.5	25.4	30.1
Case 2	27 years	14.2	5.12	85.5	27.7	32.4

[Hb - Haemoglobin, MCH - Mean cell haemoglobin, MCV - Mean cell volume, RBC - Red blood cell count, MCHC - Mean cell haemoglobin concentration]

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Table – 2: CE-HPLC Hb chromatogram parameter.

Peak	R.time	Height	Area	Area %
Unknown	0.14	2939	6149	0.3
A1a	0.20	3525	13581	0.7
A1b	0.29	4150	15576	0.8
F	0.48	1812	9828	< 0.8 *
LA1c/CHb-2	0.69	1891	13395	0.7
A1c	0.87	6713	59444	4.9
P3	1.48	9191	60185	2.9
A0	1.74	280647	1492707	72.9
A2	2.96	7147	79005	3.2
Unknown	3.24	3516	13548	0.7
Unknown	3.78	76520	284065	13.9
Total Area:	2047482			

Concentration:	%
F	< 0.8 *
A1c	4.9

A2	3.2

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Table – 3: CE-HPLC Hb chromatogram parameter.

Peak	R.time	Height	Area	Area %
Unknown	0.14	4997	9501	0.3
A1a	0.19	4976	13111	0.5
A1b	0.28	9324	39830	1.4
LA1c/CHb-2	0.72	1807	12422	0.4
A1c	0.86	7790	71586	4.6
P3	1.50	10596	76284	2.7
A0	1.73	342297	2091508	73.8
A2	2.98	9504	91453	2.7
Unknown	3.26	4064	19189	0.7
Unknown	3.81	94327	407689	14.4
Total Area:	2832572			

Concentration:	%
A1c	4.6
A2	2.7

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Photo – 1: Elution pattern showing the Hb Q India variant haemoglobin with the Bio-Rad cation exchange HPLC.

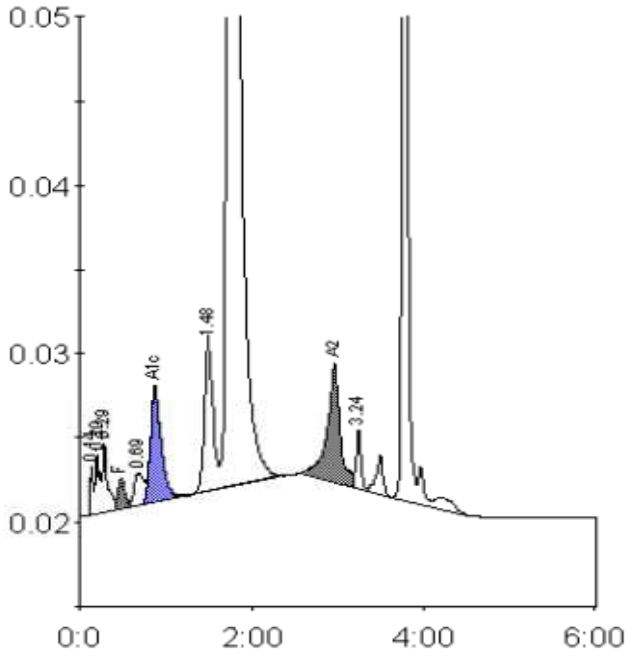
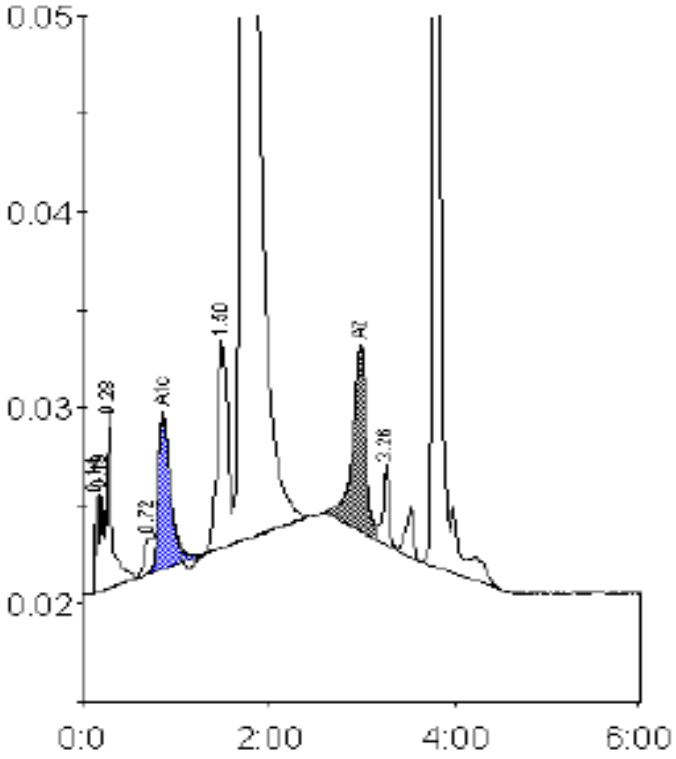


Photo – 2: Elution pattern showing the Hb Q India variant haemoglobin with the Bio-Rad cation exchange HPLC.



Source of support: Nil **Conflict of interest:** None declared.