ORIGINAL RESEARCH PAPER

Pathology

A CASE REPORTS OF RARE CO-EXISTENCE OF HB Q INDIA & BETA-THALASSAEMIA TRAIT FOUND DURING FAMILY SCREENING.

KEY WORDS: Hemoglobin Q India, Beta Thalassemia, HPLC, Antenatal Screening Test.

Dr. Sorathiya Varsha P.* Indian Medical Scientific Research Foundation Life Blood Centre, 24-vijay Plot, Samir Dholakiya Marg, Malaviya Road, Rajkot – 360002, Gujarat, India *Corresponding Author

Dr. Vachhani Nishith A. Indian Medical Scientific Research Foundation Life Blood Centre, 24-vijay Plot, Samir Dholakiya Marg, Malaviya Road, Rajkot – 360002, Gujarat, India

Dr. Nandani Sanjeev L. Indian Medical Scientific Research Foundation Life Blood Centre, 24-vijay Plot, Samir Dholakiya Marg, Malaviya Road, Rajkot – 360002, Gujarat, India

Ms. Vekariya Daya J. Indian Medical Scientific Research Foundation Life Blood Centre, 24-vijay Plot, Samir Dholakiya Marg, Malaviya Road, Rajkot – 360002, Gujarat, India

Mrs. Kashiyani Heena N. Indian Medical Scientific Research Foundation Life Blood Centre, 24-vijay Plot, Samir Dholakiya Marg, Malaviya Road, Rajkot – 360002, Gujarat, India

ABSTRACT

Hemoglobin Q India (α 64 Asp→His) is a very rare alpha chain structural variant caused by mutation in the position of codon 64 of alpha 1 gene. Usually it presents in heterozygous state and with normal haematological blood picture. India is known as a country with a high prevalence of various haemoglobinopathy. We have reported two cases of Hb Q India with beta thalassemia trait during antenatal thalassemia screening covering her Family. Haemoglobin estimation was performed by automated cell counter & haemoglobin variant analysis was done by HPLC method. Parents with various heterozygous states can lead to offspring with double heterozygous or homozygous defects. So, advisable to do careful antenatal screening test to diagnose this rare condition.

INTRODUCTION :

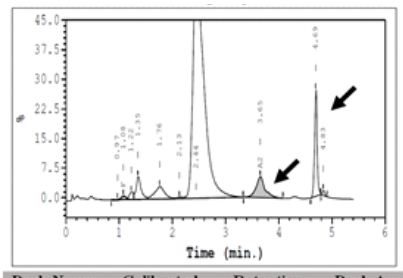
The inherited hemoglobin disorders are the most common single gene defect in human.[1] India is a country with a high prevalence of haemoglobinopathy. Hb Q has three structural variants. These include Hb Q Thailand (α 74 Asp→His), Hb Q Iran (α 75 Asp→His) & Hb Q India (α 64 Asp→His). Hb Q India is a very rare alpha chain structural variant caused by mutation AAG→CAG Asp→His in the position of codon 64 of alpha 1 gene characterized by the replacement of aspartic acid by histidine.[2] Hb Q was first described by Vella *et al*. in 1985 in association with α thalassemia in a Chinese family.[7] The first case of Hb Q India was reported by Sukumaran[8] in 1972 in a Sindhi family with associated β Thalassemia & later by Desai.[9]

Usually Hb Q India is clinically silent with normal blood picture & is generally incidentally detected during population or family screening.[4, 10, 11,] Even its presence with beta thalassemia trait does not produce any clinical abnormality & patients are mostly asymptomatic.[10] But, rare combination of Hb Q India & Hb H may be more symptomatic.[12] This Hb Q variant has normal solubility.[3] The prevalence of Hb Q India in India is 0.4%, found predominantly in sindhi families. Hb Q India levels in heterozygotes are normally below 20% and reduce further in interactions with β thalassemia.[4] This prevalence of Hb Q India was found to be lower than Hb S, Hb E, & Hb D Punjab specially in Indian population.[13] Molecular characterization of hemoglobin variants is usually done at two levels. The first level involves gel electrophoresis or cation exchange high performance liquid chromatography (CE-HPLC) while the second level of analysis engages Mass Spectrometry (MS) and/or DNA sequencing.[5,6] 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.

CASE STUDIES :

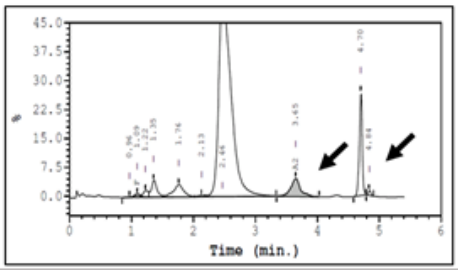
A 27 years old lady from Obs.&Gyn. OPD came for Thalassaemia testing at IMSRF. Whenever any case was detected positive for a hemoglobin disorder, partner screening was done and the couple was counselled accordingly. Blood samples collected in EDTA tube was first analysed for a routine CBC by sysmax XP-100 & then subjected for HPLC in Bio-Rad variant II as per manufacturer's guidelines. The report showed her to be beta thalassaemia trait. Advised her to have spouse HPLC testing. Her Husband's sample was taken and result was analysed. Her husband's HPLC report showed characteristic retention time of 4.69min. which showed this abnormal Hb variant was Hb Q India & HbA_2 value >4.0% indicates the diagnosis of beta thalassemia trait. So the interpretation given was double heterozygous for Hb Q India & Beta Thalassaemia trait. (Case-1) (Figure-1)

Figure-1:CE-HPLC chromatogram from Bio-Rad Variant II showing peak at 3.65 min.(Beta thalassemia trait) & 4.69 Min.(Hb Q India)



Peak Name	Calibrated area%	Retention Time (Min.)	Peak Area
Unknown	0.0	0.97	1063
F	0.6	1.08	17023
Unknown	1.0	1.22	27769
P2	3.4	1.35	94238
P3	3.8	1.76	105040
Unknown	0.3	2.13	7509
Ao	76.7	2.44	2114743
A2	5.4*	3.65	159116
Unknown	8.0	4.69	220821
Unknown	0.3	4.83	8768
		Total area: 2756090	

Figure-2: CE-HPLC chromatogram from Bio-Rad Variant II showing peak at 3.65 min.(Beta thalassemia trait) & 4.70 Min.(Hb Q India)



Peak Name	Calibrated Area%	Retention Time (Min.)	Peak Area
Unknown	0.0	0.96	796
F	0.6	1.09	15209
Unknown	1.1	1.22	26197
P2	2.8	1.35	66556
P3	3.9	1.76	91892
Unknown	0.3	2.13	6559
Ao	76.6	2.46	1788560
A2	4.7	3.65	124013
Unknown	8.9	4.70	207614
Unknown	0.4	4.84	9039
		Total area :	2336435

Result of her husband's father Sample was NORMAL & her husband's mother showed the report as double heterozygous for Hb Q India & beta thalassaemia.(Case-2)(Figure-2)

Table-1 provides hematological parameters of both double heterozygous cases of Hb Q India & beta thalassemia trait.Case-1 showed no anaemia but reduced RBC indices. While case-2 showed anaemia with microcytic blood picture& reduced RBC indices. Low RBC indices in both the cases was due to co-inheritance of β thalassemia trait.

Table-1: Hematological parameters

	Age	Hb (g/dl)	RBC (x1012/l)	MCV (fl)	MCH (pg)
Case-1 (male)	26 years	13.4	6.09	57.0	22.0
Case-2 (female)	41 years	10.3	4.94	65.0	20.9

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

CONCLUSIONS :

Nowadays various methods are available for identification of hemoglobin variants, but the most confirmatory method is DNA sequencing of the α and β globin genes. But it is very expensive technique & not practical for the routine identi fication of rare variants. Because most α and β chain variants results from single point mutation. We stress on the point that in a developing country like India simple, rapid & inexpensive method like CE-HPLC can be the basis of identification of rare hemoglobinopathies. The results showed here prove that CE-HPLC method can form the basis of a new routine diagnostic strategy for the rare abnormal hemoglobinopathies. The most effective approach to reduce the burden and to prevent thalassaemia in the society is implementation of combi nations of programmes like thalassaemia carrier screening, antenatal screening & awareness programmes.

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