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Case Report

Role of HPLC in the diagnosis of Hemoglobin Q Disease

Amrit Kaur Kaler[1*@], Viswanath Veeranna[2**], Uma Bai[1***], Raja P[1***], Parminder Kaur[3]

[*]Assistant Professor, [**]Professor, [***]Associate Professor
[1]Dept. of Pathology, MVJ Medical College and Research Hospital, Bangalore,
[2]Dept. of Pathology, Indira Gandhi Institute of Child Health,
[3]Intern, MVJ Medical College and Research Hospital, Bangalore.

[@]Correspondence Email: amrit_kaler@yahoo.co.in

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ABSTRACT

Hemoglobin Q Disease (HbQ-India) is a rare alpha globin chain variant and usually presents in the heterozygous state. It becomes symptomatic only in a homozygote state and when present in association with other conditions like beta-thalassaemia, alpha-thalassaemia, HbE and HbH. Majority of the centres in India use conventional methods for diagnosis of haemoglobinopathies, which includes clinical and family history, complete blood counts (CBC), red cell indices, RDW, HbA2, HbF estimation, sickling test and Hemoglobin electrophoresis. The limitations of these tests include identification of Hemoglobin (Hb) variants with same electrophoretic mobility (HbQ, HbG, HbD), in diagnosis of HbS traits and where low quantity of HbS is associated with negative sickling test and diagnosing certain compound heterozygous states (HbS – beta thalassaemia, HbS – HbD disease). HPLC has been shown to be rapid, sensitive, specific and reproducible alternative to conventional haemoglobin electrophoresis.

We describe a case of 35 year old male, diagnosed probably as HbS/HbD on gel electrophoresis and negative sickling test, showed a retention time of 4.76 minutes with an intense peak for this abnormal Hb variant consistent with HbQ-India.

Key words: HPLC, Hemoglobin Q Disease, structural variant, alpha Thalassemia

INTRODUCTION

Hemoglobin Q – India is a rare alpha chain variant caused by the mutation AAG-->GAG (Asp-->His) in the position of codon 64 of the alpha1 gene. [1] HbQ was first described by Vella et al in association with alpha thalassaemia in a Chinese patient. [2] HbQ India is usually seen in the heterozygous form which is clinically silent. The identification of haemoglobin variants by conventional techniques are often presumptive, based on ethnic origin of the parents and the quantification of electrophoretic mobility of the band. [3] Therefore, careful screening of the samples using routine techniques like Hb electrophoresis and HPLC are needed for identification of such abnormal hemoglobin variants.

We present a rare case of heterozygous HbQ- India, which was picked up on routine population screening of Sikh population in Bangalore.

CASE HISTORY

In a population based screening study for haemoglobinopathies in the Sikh community, Bangalore, a 35 year old male subject was detected to have an abnormal Hb (approximately 16.6%)at the position of HbS/D/G by agar gel electrophoresis (EPH) at alkaline pH. Automated haematology analyser showed no abnormality in hemoglobin and RBC indices. Peripheral smear showed predominately target cells. (as shown in figure no. 1) Sickling test was found to be negative. The patient was asymptomatic without any significant past or family history. He was hence suspected to have Hemoglobin D Punjab as HbD is known to migrate along with HbS region on alkaline EPH. (as shown in figure no. 2) To confirm HbD, the sample was subjected to analysis by high-performance liquid chromatography (HPLC). The analysis showed a retention time of 4.76 minutes with an intense peak for this abnormal Hb variant consistent with HbQ-India. HbQ was quantified to be 16.6% which is in conformity with heterozygous state of HbQ. (as shown in figure no. 3) Later, his brother and 2 daughters were also screened and his brother and one daughter was found to be positive for HbQ-India.

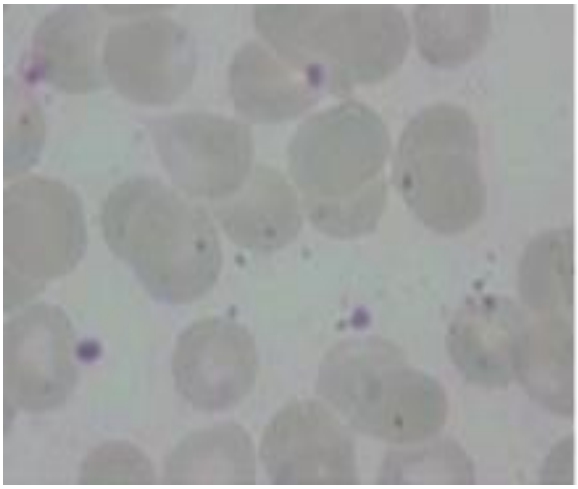


Fig 1: Peripheral smear shows predominately tear cells.

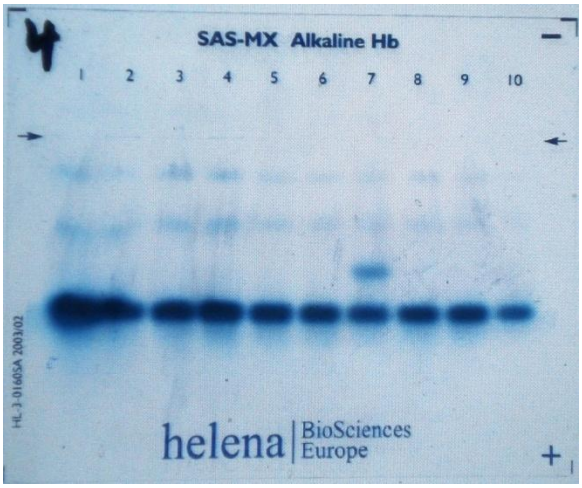
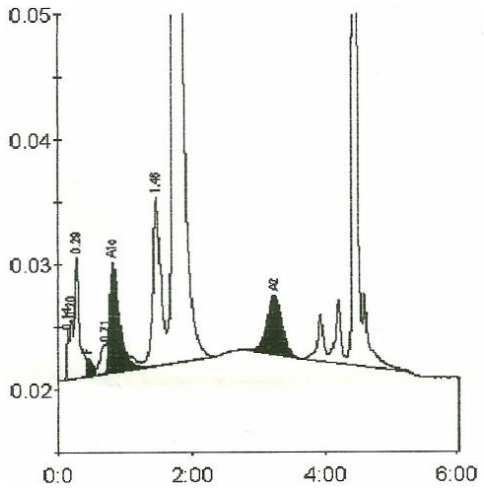


Fig 2: Gel electrophoresis shows a band at HbS/HbD/HbG/HbQ region.



Peak	R.time	Height	Area	Area %
Unknown	0.14	4026	10102	0.4
A1a	0.20	4764	15692	0.6
A1b	0.29	9698	44086	1.6
F	0.45	1453	8301	< 0.8 *
LA1c/CHb-1	0.71	2333	16952	0.6
A1c	0.83	8614	85677	5.0
P3	1.46	13421	110920	4.0
A0	1.75	356978	1957404	70.2
A2	3.24	4758	74495	2.6
Unknown	4.43	116733	463852	16.6
Total Area:			2787480	

Concentration:	
% F	< 0.8 *
% A1c	5.0
% A2	2.6

HbQ - India

Fig 3: The graph shows an intense peak with retention time of 4.76 minutes consistent with HbQ-India.

DISCUSSION

The first case of HbQ India was described by Sukumuran in 1972 in a Sindhi family in association with beta thalassemia and later by Desai et al. [4,5] HbQ variants are formed due to structural mutations at the alpha globin chain and are clinically silent. This is because the mutation involved α64 (E 13), is on the surface of the hemoglobin tetramer and the charge changes at these positions do not affect the properties of the hemoglobin molecule. [6] Three molecular structural variants have been documented in the literature, namely HbQ-India (alpha 64 Asp to His), HbQ-Thailand (alpha 74 Asp to His) and HbQ-Iran (alpha 75 Asp to His).

The quantity of the HbQ variant is determined by the ratio of alpha A, alpha Q and beta A globin chains. HbQ-India is known to be affected by the presence of other haemoglobinopathies. The presence of concomitant alpha-thalassemia favors the formation of HbQ, whereas beta-thalassaemia reduces the formation of HbQ. This has been explained to be due to a post-translational control mechanism. [7] HbQ disease in its homozygous state is characterized by presence of HbQ levels of about 35%, where as in heterozygous state it is in the range of 20%. [8] In the present case, HbQ levels were estimated to 16.6%, hence fitting into heterozygous state.

The most common investigative tools for diagnosis of haemoglobinopathies are HbF quantification by alkaline denaturation method, HbA2 quantification by ion – exchange column chromatography and alkaline and acid gel electrophoresis for haemoglobin. [3] It is important to identify various clinically significant variants with varying degrees of severity, like unusual variants (HbQ India, HbD Punjab, HbD Iran) or compound heterozygous disorders (HbSD – Punjab, HbSE, HbS - thalassaemia). [9,10] None of these can be conclusively identified by a single electrophoretic technique. [11]

Tyagi et al has quoted that HbQ in earlier times has been described as HbD by many authors. [12] It becomes symptomatic when seen in association with other diseases. On agar gel EPH at alkaline pH, the HbQ band can easily be misinterpreted as HbS/HbD/HbG if careful screening of the patient is not done. HbS can be ruled out based on family history and solubility test or sickling test for sickle cells. It is difficult to differentiate between HbQ/HbD, HbG in heterozygous state based on routine electrophoresis.

HPLC offers the distinct advantage over classic haemoglobin electrophoresis as it can more accurately identify and quantitative abnormal haemoglobins. It is also very useful for paediatric group of patients, as only 5µl of blood is sufficient for analysis. [3] The disadvantage of HPLC variant is that it requires skill in interpretation. Also, various normal and abnormal haemoglobins may have same retention time; the glycosylated variant Hb analyzer may have different retention time from non glycosylated variant Hb analyzer. HbE and Hb Lepore coelute with Hb A2 move at the same range. [3] Isoelectric focusing as a second line technique can resolve the issue.

The mutation can also be identified with sequencing of the concerned region by ARMS-PCR, which demonstrates the specific location of the mutation of HbQ-India. [6]

CONCLUSION

As consanguineous marriages are common in India, it is essential to undergo screening and genetic counselling to prevent the occurrence of homozygous HbQ disease. It is thus recommended that any hemoglobin which migrates to 'S' region on agar gel EPH in alkaline EPH, HPLC should be

definitely be performed for further sub characterization of rare Hb variants. HPLC is a cheaper alternative to ARMS-PCR in detection of the rare haemoglobinopathies.

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