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Original Research Article

HBQ-India: an uncommon hemoglobin variant

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ABSTRACT

Background: HbQ-India is a rare alpha chain variant. It is an important member of the hemoglobin Q family molecularly characterized by replacement of aspartic acid by histidine. It usually presents in the heterozygous state. It becomes symptomatic only in the homozygous state and when present in association with other conditions like beta-thalassaemia, alpha thalassaemia, HbE and HbH. The Sindhi is one of the largest linguistic communities, migrated about 65 years back from the Sindh province of west Pakistan to India. They are a high-risk community for beta thalassaemia gene in India with a carrier frequency ranging from 5 to 12 % with a distinct regional variability.
Methods: A total 343 cases were screened for hemoglobinopathies in Sindhi population. Detail history was taken from each patient and pertinent physical finding were noted. CBC, Peripheral smear and HPLC were performed. During screening we observed that few samples showed an unknown peak at a retention time of 4.7 min on HPLC and comparison with reference chromatograms indicated them to be HbQ India and it is confirmed by amplification restriction mutation system polymerase chain reaction (ARMS-PCR).
Results: We found 13 cases, 12 cases of HbQ India and 1 case of HbQ-beta thalassaemia in Sindhi population of Aurangabad in Maharashtra.
Conclusions: India is known as a country with a high prevalence of different types of hemoglobinopathy. Now a days HPLC, IEF, ARMS-PCR, DNA sequencing are the methods available for the diagnosis of the abnormal Hb like HbQ-India. HPLC is a cheaper alternative to ARMS-PCR in the detection of rare hemoglobinopathies.

Keywords: Alpha thalassaemia, Hemoglobin Q, HPLC, PCR

INTRODUCTION

Hemoglobin has plenty of variants.[1] Hemoglobin (HbQ) is a clinically silent hemoglobinopathy that results from modification of alpha 1 globin gene (AAG>GAG) substituting arginine for histidine. HbQ was first described by vella et al, in association with alpha thalassaemia in a Chinese patient.[2] Three variations have been described namely India, Thailand and Iran based on the involved codon (64,74 and 75 respectively).[3] This infrequent disorder has been detected in the homozygous and heterozygous states in association with alpha and beta thalassaemia.

The first case of HbQ-India was reported by Sukumaran in 1972 in a Sindhi family with associated beta thalassaemia and later by Desai.[4,5] HbQ-India is usually seen in the heterozygous form which is clinically silent not having deleterious phenotypic effect.[4] The major clinical significance of HbQ is its correct diagnosis. The identification of hemoglobin variants by conventional techniques are often presumptive, based on ethnic origin of the parents and the quantification of electrophoretic mobility of the band.[6]

On alkaline electrophoresis, HbQ has the same mobility as hemoglobin S/D, Hence Hb F and A2 with alpha chain

lead to appearance of accessory bands corresponding to abnormal hemoglobin thus making the diagnosis of HbQ rather cumbersome.[5] Recently many laboratories have adopted high performance liquid chromatography (HPLC) as their primary mode of screening and the reason for this choice may be the case, cost effectiveness or correct diagnosis for various hemoglobinopathies.

Here by we report total 13 cases, 12 cases of HbQ-India and 1 case of HbQ-beta thalassaemia, which were picked up on routine population screening of Sindhi population in Aurangabad in Maharashtra.

During screening we observed that few samples showed an unknown peak at a retention time of 4.7min on HPLC and comparison with reference chromatograms indicated them to be HbQ India. With the availability of DNA analysis, we were able to characterize them at the molecular level.

METHODS

This is a population-based screening study for hemoglobinopathies in sindhi population at Aurangabad in Maharashtra. It is a retrospective study carried out in a tertiary care hospital for three years. A total 343 cases were screened. Detail history was taken from each patient and pertinent physical finding were noted.

An 2 ml blood in EDTA from each patient was taken. CBC was performed using an automated hematology analyzer. Peripheral film was examined. The variant beta thalassaemia short program that utilizes the principle of cation exchange high performance liquid chromatography was used for detection of hemoglobin variants according to the manufacturer's instructions.

Chromatograms with unknown peak at a retention time of 4.7 min were selected and compared with reference chromatograms. It indicated to be HbQ India. HbQ was quantified to be 16.6% which is in confirmity with heterozygous state of HbQ. All the cases were confirmed by AMRS-PCR.

RESULTS

A total 343 cases were screened for hemoglobinopathies in Sindhi population. This study was carried out for 3 years in a tertiary care hospital. We identified 12 cases of HbQ India and one case of HbQ-beta thalassaemia during this duration. Other hemoglobinopathies that were detected were beta thalassaemia trait, beta thalassaemia major, sickle cell trait and Hb G Philadelphia.

Out of the 12 cases of HbQ India, 9 were males and 3 were females with age ranging from 3 to 60 years (Mean 27.3 years). Table 1 shows the age and sex of all the cases. All these cases were picked on routine Sindhi population screening. All of them were asymptomatic. Physical examination also did not reveal any significant finding. All the 3 females had mild anemia and peripheral smear revealed normocytic mild hypo chromic anemia. Only 2 males out of 9 males showed mild anemia with normocytic normchromic blood picture. The red cell distribution width was normal in all the patients. All the patients had normal white cell count and platelet count.

Table 1: Presentation of patients with hemoglobin q india (n=13).

Patients	Ethnicity	Age (years)	Sex	Reason of HPLC	Physical findings
1	Sindhi	38	M	Routine screening	NIL
2	Sindhi	12	F	Routine screening	NIL
3	Sindhi	14	M	Routine screening	NIL
4	Sindhi	3	M	Routine screening	NIL
5	Sindhi	10	F	Routine screening	NIL
6	Sindhi	6	M	Routine screening	NIL
7	Sindhi	30	M	Routine screening	NIL
8	Sindhi	60	M	Routine screening	NIL
9	Sindhi	30	M	Routine screening	NIL
10	Sindhi	55	M	Routine screening	NIL
11	Sindhi	34	F	Routine screening	NIL
12	Sindhi	36	M	Routine screening	NIL
13	Sindhi	10	M	Routine screening	NIL

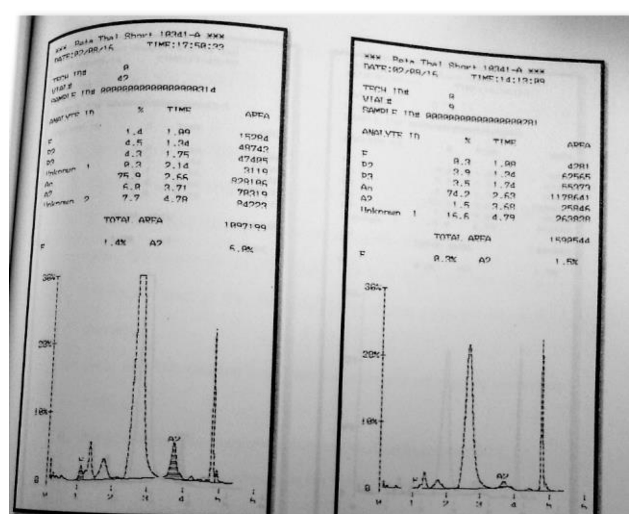

Figure 1: Hemoglobin separation obtained by automated HPLC in patient with HB Q (right) and compound heterozygosity for HB Q india and beta thalassemia trait (left).

HbQ was identified by an abnormal peak at a retention time of 4.79 min in a chromatogram (Figure 1). Results of HPLC are summarized in Table 2.

We had one male of 10 years who was diagnosed as HbQ-beta thalassaemia. On routine screening, he had anemia and the indices showed presence of thalassaemic indices. The solubility test for sickling was negative. HPLC showed an abnormal peak with a retention time of 4.78 min and HbA2 levels of 6% (Figure 1). This confirmed the concomitant presence of HbQ-India with beta thalassaemia trait. Family study was carried out which showed father to be HbQ-India and mother to be beta thalassaemia trait.

Table 2: Hemogram and chromatographic analysis with patients with hemoglobin Q India (n=13).

Patients	Hb (g/dl)	RBC (10^{12}/L)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	Hb A (%)	HbA2 (%)	Hb F (%)	Hb X (%)	Retention time (min)
M	15.2	5.36	48.3	90.1	28.4	31.5	13.5	74.2	1.5	0.3	16.6	4.79
F	11.6	4.80	38.8	74.8	22.6	30.6	13.6	73.4	1.3	0.2	17.4	4.79
M	14.1	4.44	44.4	100	31.8	31.8	14	73.5	1.7	0.4	17.0	4.79
M	12.1	5.06	36.4	71.9	23.9	33.2	14	72.9	1.6	0.5	15.7	4.79
F	11.7	5.19	38.7	74.6	22.5	30.2	15.6	71.5	1.4	0.5	17.6	4.79
M	12.0	4.82	36.4	75.5	24.9	33.0	14.1	71.1	1.6	0.2	16.5	4.79
M	15	5.39	46.3	85.9	27.8	32.4	13.2	74.4	1.2	0.2	16.0	4.79
M	13.9	4.80	43.7	91.0	29.0	31.8	13.7	72.4	3.8	0.2	16.8	4.79
M	14	5.29	43.6	83.7	26.9	32.1	13.3	76.1	1.2	0.1	15.6	4.79
M	13.2	5.05	41.9	83.0	26.1	31.5	13.2	72.1	3.8	0.2	16.5	4.77
F	10.7	4.39	35.9	81.8	24.4	29.8	15.5	73.5	3.8	0.2	16.2	4.77
M	14.1	4.46	44.3	88.4	23.2	31.8	13.6	74.4	1.2	0.2	16.0	4.79
M	10.3	5.39	33.5	62.2	19.1	30.7	15.3	75.9	6	1.4	7.7	4.78

DISCUSSION

The first case of HbQ India was described by Sukumaran in 1972 in a Sindhi family in association with beta thalassaemia 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 alpha 64 is on the surface of hemoglobin tetramer and the charge changes at these positions do not affect the properties of hemoglobin molecule.[7] Three molecular variant types have been documented namely HbQ-India, HbQ-Thailand and HbQ-Iran. The prevalence of HbQ-India in India is 0.4% found predominantly in Sindhi families and in individuals from Western and Northern India.

Normally, HbQ is clinically silent. As the hemoglobin is not altered structurally at its tertiary level, the presence of HbQ does not impart any functional deficit and hence lacks the clinical manifestation for that matter.[4,8] Even presence of HbQ along with beta thalassaemia does not seem to produce clinical abnormality. But HbQ-H can give clinical manifestations though it is very rare.[9] In our study, all the cases were asymptomatic. The quantity of 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 hemoglobinopathies. The presence of concomitant alpha-thalassaemia favours the formation of HbQ, whereas beta thalassaemia reduces the formation of HbQ. This has been explained due to post translation control mechanism.[10] HbQ disease in its homozygous state is characterized by presence of HbQ levels of about 35% whereas in heterozygous state it is in the range of 17 to 20 % and further reduce in interaction with beta thalassaemia.[11] In our study HbQ levels were estimated to be 16.33+/-0.60, hence fitting into the heterozygous state. The most common investigative tools for diagnosis of hemoglobinopathies are HbF quantification by alkali denaturation method, HbF quantification by ion exchange column chromatography.

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 P^H, 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 the 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 clinical advantage over classic hemoglobin electrophoresis as it can more accurately identify and quantify abnormal hemoglobins. It is also

useful in paediatric patients as only 5ul of blood is sufficient for analysis.[6] The HbQ disorder is readily detected by HPLC as HbQ gets eluted at 4.7 minute of analytic time.

This 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.[7] We were also able to characterize HbQ India at the molecular level using ARMS-PCR.

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. Now a days HPLC, IEF, ARMS-PCR, DNA sequencing are the methods available for the diagnosis of the abnormal Hb like HbQ-India.

As consanguineous marriages are common in India, it is essential to undergo screening and genetic counselling to prevent the occurrence of homozygous HbQ disease. Thus, careful screening of the samples should be done. HPLC can be the basis of identification of abnormal hemoglobin variants like HbQ. HPLC is a cheaper alternative to ARMS-PCR in the detection of rare hemoglobinopathies.

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REFERENCES

1. Rathod G, Aggarwal S, Goyal R, Patel R, Bhimani RK, Kuchhal NK. Rare Haemoglobin Variant Hb J Meerut in 27 Years Old Female-A Case Report. 2014.
2. Vella F, Wells RHC, Ager JAM, Lehmann H. A hemoglobinopathy involving hemoglobin H disease and new Q disease. Br Med J. 1958;1:725-7.
3. Wiwanitkit V. Phylogenetic tree of hemoglobin Q disorders. The Internet J Hematology. 2004;2(1).
4. Sukumaran PK, Merchant SM, Desai MP, Wiltshire BG, Lehmann H. Haemoglobin Q India (alpha 64 (E13) aspartic acid histidine) associated with beta-thalassemia observed in three Sindhi families. J medical genetics. 1972;9(4):436.
5. Desai DV, Dhanani H, Kapoor AK, Yeluri SV. Hb Q-India in a Sindhi family: An unknown hemoglobin variant. Lab Hematol. 2004;10:212-4.
6. Bhat VS, Dewan KK, Krishnaswamy PR, Mandal AK, Balaram P. Characterization of a hemoglobin variant: HbQ-India/IVS 1-1 [G> T]-β-thalassemia. Ind J Clin Biochem. 2010;25(1):99-104.
7. Abraham R, Thomas M, Britt R, Fisher C, Old J. Hb Q-India: an uncommon variant diagnosed in three Punjabi patients with diabetes is identified by a novel DNA analysis test. J Clin Pathol. 2003;56:296-9.
8. Wiwanitkit V. Secondary and tertiary structure aberration of alpha globin chain in haemoglobin Q-India disorder. Indian J Pathology Microbiol. 2006;49(4):491-4.
9. Taj J, Tay JS, Wong YC, Kham SK, Bte Abd Aziz N, Teo SH, et al. Molecular analysis of Hb Q-H disease and Hb Q E in a Singaporean family. Southeast Asian J Trop Med Public Health. 1995;26 Suppl 1:252-6.
10. Qin WB, Baysal E, Wong KF, Molchanova TP, Pobedimskaya DD, Sharma S, et al. Quantities of αQ chain variants in heterozygotes with and without a concomitant β-thalassemia trait. Am J Hematology. 1994;45(1):91-3.
11. Phanasgaonkar, S. Colah R, Ghosh K, Mohanty D, Gupte S. Hb Q-India and its interaction with β-thalassaemia: a study of 64 cases from India. Brit J Biomed Sci. 2007;64(4):160-3.
12. Tyagi S, Saxena R, Choudhry VP. HPLC-how necessary is it for haemoglobinopathy diagnosis in India?. Ind J Pathol Microbiol. 2003;46(3):390-3.

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