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# Incidental detection of HbQ-India during beta-thalassemia screening

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## Abstract

HbQ-India is a relatively rare hemoglobin variant seen particularly in the Indian subcontinent. In this report we present twenty cases of HbQ-India variant and two cases of HbQ India/Beta- thalassemia detected in our lab during thalassemia screening tests conducted in camps along with family studies of few cases. These cases were detected over a span of three months from samples screened for thalassemia. In order to perform familial studies, sample of family members of six cases were collected totaling to twenty-two affected cases. Molecular confirmation of the detected HbQ-India trait was done using PCR-ARMS technology. Our study detected frequency of HbQ-India carriers at 1.47%.

Key words : Hemoglobinopathy, HbQ-India, frequency.

## INTRODUCTION

Hemoglobin is the oxygen carrying partner in RBC and has four globin chains *viz.* two alpha and two beta each. Hemoglobinopathies include an array of disorders caused due to mutations in the alpha and beta chain of hemoglobin. In new born children, the percentage of fetal hemoglobin or HbF ($\alpha 2\gamma 2$) is highest but as aging happens, a switch over occurs from the $\gamma$ chain to $\beta$ chain synthesis. In adults the percentage of HbA ($\alpha 2\beta 2$) is highest at ~95% with minor fraction of HbA2 ($\alpha 2\delta 2$) at <3.5% and HbF at <2%. The presence of common beta chain variants like HbS, HbE, HbD Punjab or beta thalassemia mutations may lead to very severe sickle cell disease or beta thalassemia major in the progeny of healthy carrier couples. HbQ-India is a clinical condition which develops due to a genetic alteration 64 (E13) Asp>His in the alpha 1 gene also termed the HBA1:c.193G>C variant.

### Case History

Thalassemia screening is done as part of routine diagnostic test in our lab. Screening camps for thalassemia are regularly organized by Thyrocare Technologies Limited. Initial screening of samples were done from camps organized in three locations *viz.* Bhusawal, Wadsa and Lucknow from Central part of India. Sixteen healthy carriers of HbQ-India trait were detected from these camps within a period of three months from a total of 1497 samples screened. Family studies of six detected carriers were done leading to final reporting of twenty-two cases. Twenty of the twenty-two detected carriers were completely asymptomatic and showed a normal hemogram profile while two cases were detected to be compound heterozygous for HbQ-India/Beta-thalassemia (Figures 1 and 2 represent the two cases identified).

The samples were processed for thalassemia screening using an automated HPLC analyzer calibrated for variant analysis (TOSHO G8). In case of HPLC, the HbQ variant fraction elutes in the S window in the same retention time (RT). The chromatogram of a sample detected as HbQ-India is shown in Figure 3 (Chromatogram showing the HbQ fraction as 21.6% at RT 4.68 min) while another case with microcytic anemia picture is as shown in Figure 4 (HbQ - 18.3%, RT-4.62 min). Complete blood count for fifteen cases and family members of six affected case was performed using automated hematology analyzer SYSMEX XN-1000 (Data of the RBC indices documented in Table 1). The percentage of each hemoglobin fraction for all the twenty-two healthy carriers is as shown in Table 2, while the blood count indices for the same are documented in Table 1. A detailed family tree has also been supplied for all the cases wherein familial study has been done for better understanding of transmission of the syndrome (Figure 5- a, b and c). The corresponding details about findings of each of the family is detailed in Table 1 and 2.

Molecular confirmation of two detected HbQ-India carriers were done commercially using PCR-ARMS technology from the genetics department of Sir Ganga Ram Hospital, New Delhi, India (Report attached in Figure 6).

## DISCUSSION

HbQ-India is an alpha chain variant caused due to αAsp 64 His change and is inherited as an autosomal dominant trait. Three HbQ variants namely HbQ-India, HbQ-Iran and HbQ-Thailand have been documented widely [1].The first case of HbQ-India with associated β-thalassemia was documented in a Sindhi family in 1972 [2]. The first large scale study of 64 cases of HbQ-India was documented by Phanasgaonkar et al. in the year 2007 [3]. This study documented 36 cases of HbQ-India trait, 22 cases of HbQ-Beta thalassemia and 3 cases of HbQ-Beta thalassemia major. Determination of HbQ-India trait is generally done by observing the HPLC chromatogram and determining the percentage of each of HbA, HbA2, HbF and HbQ fractions depending on the percentage reflected and the RT detected in the graph.

The sixteen HbQ-India samples detected in our lab were collected as part of thalassemia screening camps mainly organized in the central region of the country where presence of HbS, HbQ and HbD is highly documented. Many previous studies in the Indian population have already stressed on the regional concentration of hemoglobinopathies in certain geographical locations in our country [4 - 6]. Our case report

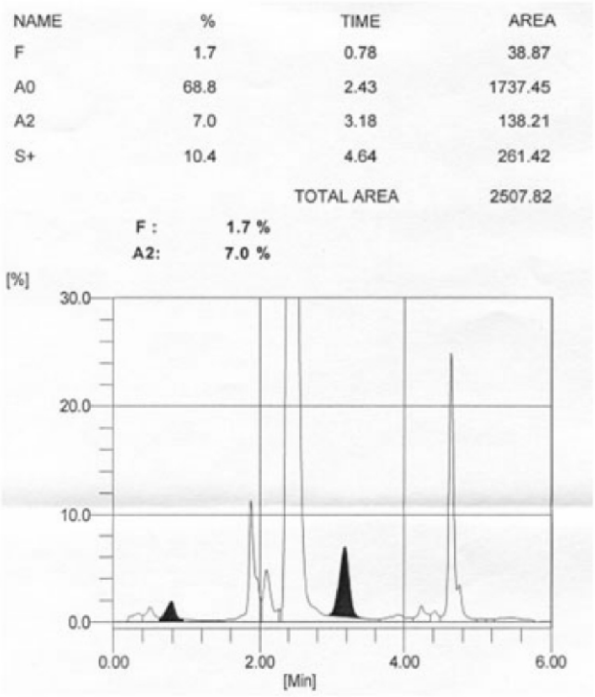



**Figure 1.** HPLC chromatogram of an HBQ-India/Beta-thalassemia case (HbQ- 10.4%, HbA2- 7%)

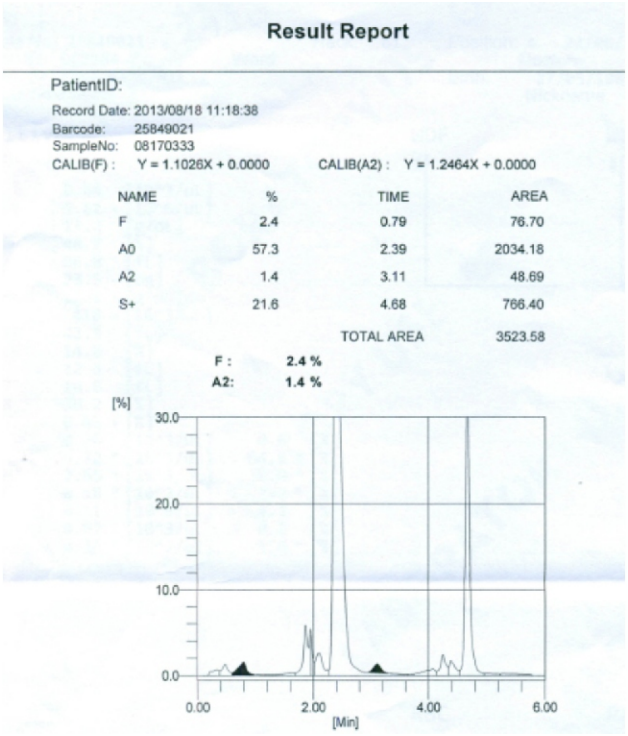



**Figure 3.** HPLC chromatogram of an asymptomatic HbQ-India carrier (HbQ- 21.6%)

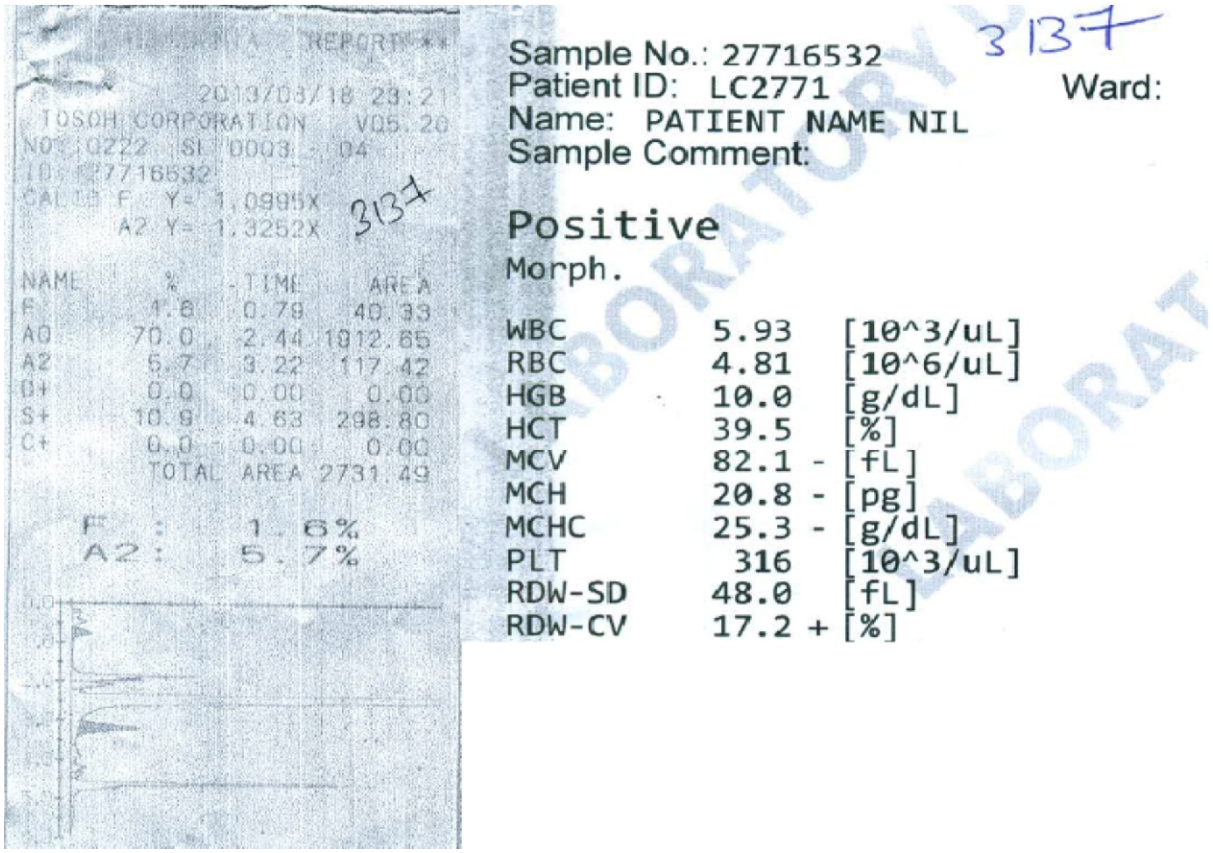



**Figure 2.** HPLC chromatogram and blood count report of an HbQ-India/Beta-thalassemia case (HbQ-10.9%, HbA2-5.7%)

**Table 1:** Summary of complete blood profile of the detected carriers for HbQ-India as well as HbQ-India/Beta-thalassemia

| Sample/ Family | Affected | RBC ($10^6$/µl) | Hb (g/dL) | MCV (fL) | MCH (pg) | MCHC (g/dL) |
|---|---|---|---|---|---|---|
| 1 | - | 4.52 | 12.7 | 95.6 | 28.1 | 29.4 |
| 2 | - | 4.82 | 12.9 | 90.5 | 26.8 | 29.6 |
| 3 | - | 5.26 | 14.1 | 87.5 | 26.8 | 30.7 |
| 4 | - | 4.81 | 10 | 82.1 | 20.8 | 25.3 |
| 5 | - | Sample not sufficient | Sample not sufficient | Sample not sufficient | Sample not sufficient | Sample not sufficient |
| 6 | - | 5.61 | 13.2 | 86.8 | 23.5 | 27.1 |
| 7 | - | 4.64 | 13.1 | 107.8 | 28.2 | 26.2 |
| 8 | - | 4.76 | 11.6 | 94.7 | 24.4 | 25.7 |
| 9 | - | 5.66 | 16.3 | 106.5 | 28.8 | 27 |
| Family 1 | Mother | 4.04 | 12 | 99 | 29.7 | 30 |
| | Son (Proband) | 4.71 | 13.5 | 97.7 | 28.7 | 29.3 |
| Family 2 | Father | 4.30 | 13.1 | 97.4 | 30.5 | 31.3 |
| | Son (Proband) | 4.19 | 8.2 | 76.4 | 19.6 | 25.6 |
| Family 3 | Mother | 4.11 | 11.8 | 95.6 | 28.7 | 30 |
| | Son (Proband) | 4.97 | 13.4 | 90.1 | 27 | 29.9 |
| Family 4 | Son (Proband) | 6.06 | 16 | 90.8 | 26.4 | 29.1 |
| | Son | 5.12 | 13.6 | 90.8 | 26.6 | 29.2 |
| Family 5 | Father | 5.17 | 14.9 | 95.9 | 28.8 | 30 |
| | Son (Proband) | 5.35 | 15 | 95 | 28 | 29.5 |
| Family 6 | Father | 4.59 | 13.9 | 98.9 | 30.3 | 30.6 |
| | Son (Proband) | 4.83 | 14.7 | 103.1 | 30.4 | 29.5 |
| | Daughter | 4.29 | 8.4 | 76.5 | 19.6 | 25.6 |

**Table 2:** Summary of hemoglobin fractions detected by HPLC chromatogram

| Sample ID | Relation | $HbA_0$ (%) RT – ~2.29 min | $HbA_2$ (%) RT – ~3.07 min | HbF (%) RT – ~0.69 min | HbQ (%) RT – ~4.60 min | Reported |
|---|---|---|---|---|---|---|
| 1 | - | 68.1 | 2 | 0.3 | 17.7 | HbQ-India |
| 2 | - | 68 | 2.2 | 0.3 | 17.8 | HbQ-India |
| 3 | - | 68.3 | 2.4 | 0.3 | 18.2 | HbQ-India |
| 4 | - | 70 | 5.7 | 1.6 | 10.9 | HbQ-India/Beta-thalassemia |
| 5 | - | 68.8 | 7.0 | 1.7 | 10.4 | HbQ-India/Beta-thalassemia |
| 6 | - | 57.3 | 1.4 | 2.4 | 21.6 | HbQ-India |
| 7 | - | 66.4 | 1.8 | 0.4 | 18.1 | HbQ-India |
| 8 | - | 68.1 | 2.0 | 0.4 | 17.8 | HbQ-India |
| 9 | - | 66 | 2 | 0.7 | 17.9 | HbQ-India |
| Family 1 | Mother | 66.6 | 2.4 | 0.3 | 19.1 | HbQ-India |
| | Son (Proband) | 66.7 | 2.0 | 0.3 | 17.6 | HbQ-India |
| Family 2 | Father | 68.6 | 2.6 | 0.3 | 18.8 | HbQ-India |
| | Son (Proband) | 66.8 | 2.5 | 0.3 | 18.3 | HbQ-India |
| Family 3 | Mother | 67.3 | 2.6 | 0.4 | 18.5 | HbQ-India |
| | Son (Proband) | 67.4 | 2.6 | 0.3 | 19.1 | HbQ-India |
| Family 4 | Mother | Not screened | Not screened | Not screened | Not screened | - |
| | Son (Proband) | 66.3 | 2.4 | 0.6 | 19 | HbQ-India |
| | Son | 66.8 | 2.3 | 0.3 | 18.6 | HbQ-India |
| Family 5 | Father | 67.4 | 2.6 | 0.4 | 18.2 | HbQ-India |
| | Son (Proband) | 66.4 | 2.6 | 0.3 | 18.4 | HbQ-India |
| Family 6 | Father | 67.8 | 2.6 | 0.3 | 18 | HbQ-India |
| | Son (Proband) | 67.4 | 2.6 | 0.6 | 18.1 | HbQ-India |
| | Daughter | 67.8 | 2.0 | 0.3 | 17.6 | HbQ-India |

RT- Retention time of the fraction in HPLC.

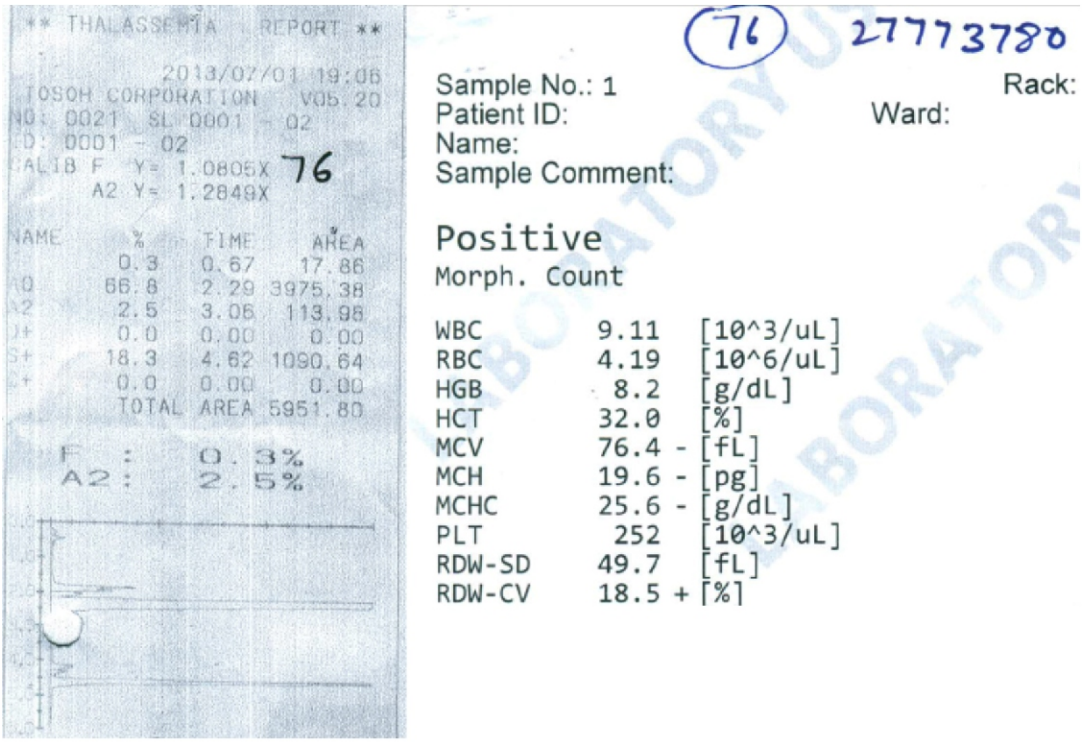
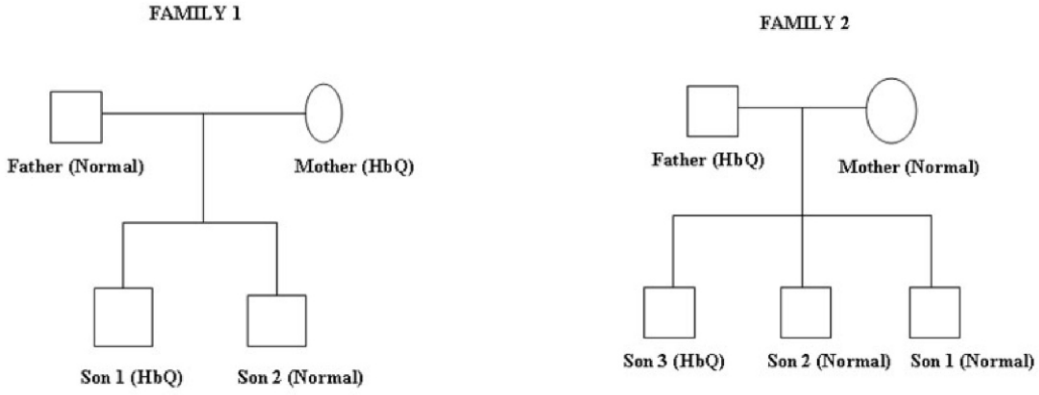


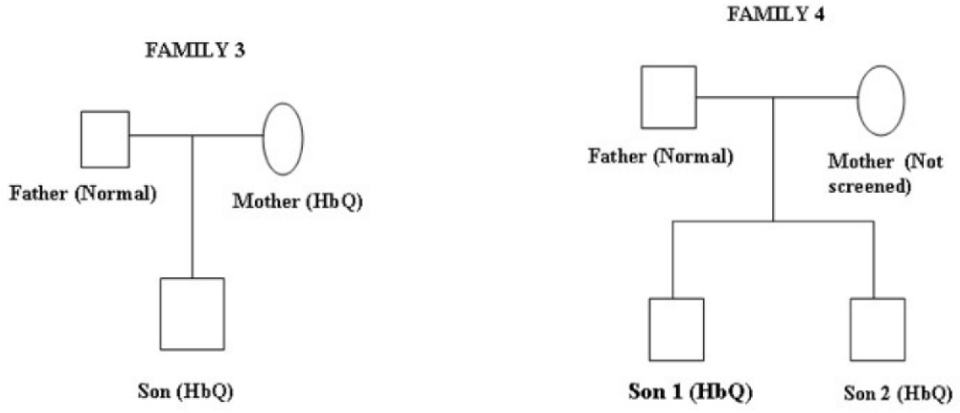

**Figure 4.** HPLC chromatogram and blood count report of an HbQ-India carrier (HbQ- 18.3%)

**Figure 5 (a, b & c):** Family tree for all the HbQ-India detected carriers to understand transmission of syndrome

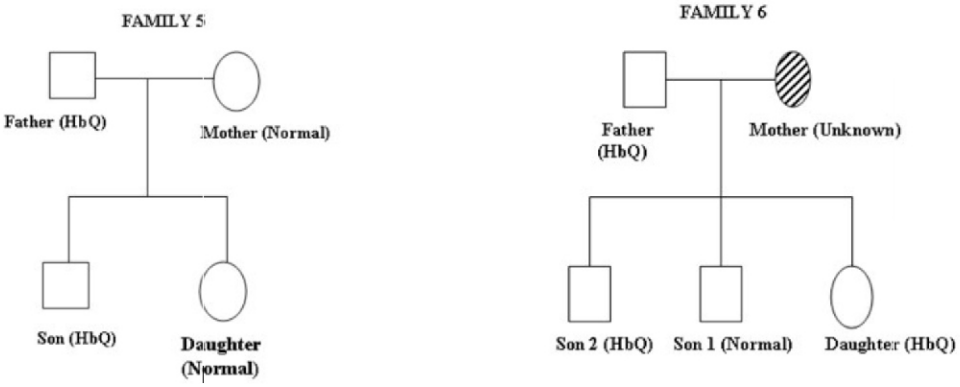
5a

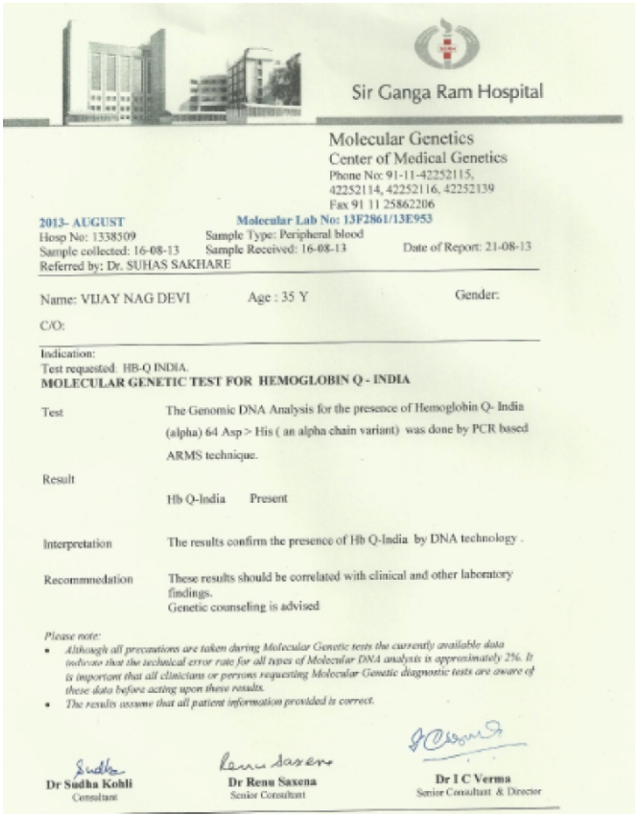



5b



5c



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2013- AUGUST
Molecular Lab No: 13F2861/13E953
Hosp No: 1338509
Sample Type: Peripheral blood
Sample collected: 16-08-13
Sample Received: 16-08-13
Date of Report: 21-08-13
Referred by: Dr. SUHAS SAKHARE

Name: VIJAY NAG DEVI Age : 35 Y Gender:
C/O:
Indication:
Test requested: HB-Q INDIA.

**MOLECULAR GENETIC TEST FOR HEMOGLOBIN Q - INDIA**

| | |
|---|---|
| Test | The Genomic DNA Analysis for the presence of Hemoglobin Q- India (alpha) 64 Asp > His ( an alpha chain variant) was done by PCR based ARMS technique. |
| Result | Hb Q-India Present |
| Interpretation | The results confirm the presence of Hb Q-India by DNA technology . |
| Recommnedation | These results should be correlated with clinical and other laboratory findings. Genetic counseling is advised |

*Please note:*
- *Although all precautions are taken during Molecular Genetic tests the currently available data indicate that the technical error rate for all types of Molecular DNA analysis is approximately 2%. It is important that all clinicians or persons requesting Molecular Genetic diagnostic tests are aware of these data before acting upon these results.*
- *The results assume that all patient information provided is correct.*

Dr Sudha Kohli, Consultant
Dr Renu Saxena, Senior Consultant
Dr I C Verma, Senior Consultant & Director

**Figure 6.** PCR confirmation report of HbQ-India case

reiterates this fact. The use of a technique like HPLC is very useful to provisionally identify common variants like HbS, HbD, HbE and beta thalassemia. A recent study by Sachdev et al. (2010) has described detection of 2600 cases of hemoglobinopathies using Bio-Rad HPLC variant analysis. This study detected presence of five cases of HbQ-India trait along with two cases of HbQ-India/Beta thalassemia double heterozygous case [7]. Another study by Rao et al. 2010 has documented the importance of cation exchange-HPLC for detecting hemoglobin variants. This study analyzed 800 samples using Bio-Rad HPLC variant system. This study also detected in case of subjects with normal electrophoretogram pattern, decrease in HbA2 levels to be associated with iron deficiency anemia and increase in the same to be associated with megaloblastic anemia [8]. However many less common variants may migrate on the same position and confirmation at the molecular level is imperative. Though molecular techniques like sequencing have been used for detection, it is very expensive and for routine diagnostic application, HPLC is by far the most robust and inexpensive for large scale applications. In our study, along with detection by HPLC, molecular confirmation was done to ascertain authentic reporting.

## CONCLUSION

Our report is one of the few to carry out large scale screening in specifically Sindhi population and document the frequency of the HbQ-India carriers at 1.47%. This case report stresses on the fact that awareness about different hemoglobinopathies must be created especially among certain communities in Indian population and such screenings must be made a part of regular pre-marital testing.

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