

## Case Report

# Accidental detection of clinically silent compound heterozygous Hb D Punjab/Hb Q India while analyzing HbA1c by high performance liquid chromatography

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## ABSTRACT

HbA1c is routinely prescribed investigations for diagnosing and monitoring diabetes and high-performance liquid chromatography (HPLC) is preferred method which is also able to identify presence of hemoglobin variant. A case was encountered where presence of variant hemoglobin was indicated. On further investigation with three different instruments, diagnosis of compound heterozygous Hb D Punjab/Hb Q India was made. The chromatogram on Bio-Rad D10 showed Hb D Punjab ( $\alpha\alpha\beta\beta^{\text{HbD Punjab}}$ )-29.89% at 3.96 minutes retention time (RT), Hb Q India ( $\alpha\alpha^{\text{HbQ India}}\beta\beta$ )-9.5% with 4.45 minutes RT, hybrid of HbQ India/Hb D Punjab ( $\alpha\alpha^{\text{HbQ India}}\beta\beta^{\text{HbD Punjab}}$ )-6% with 4.66 minutes RT, Hb A2 ( $\alpha\alpha\delta\delta$ ) was 2.5% and Hb A ( $\alpha\alpha\beta\beta$ ) was 52.2%. Analysis done on Bio-Rad variant V-II confirmed these findings. Analysis done on Sebia capillary electrophoresis revealed major peak of 50.9% in zone 9/Z(A) constituted by Hb A, second peak of 39.8% in zone 6/Z(D) constituted by co-elution of Hb D and Hb Q India, third peak of 8.8% in zone 3-4/Z(A2-C) constituted by co-elution of Hb A2 and hybrid of Hb D Punjab/Hb Q India and a fourth peak of 0.5% in zone 1 representing Hb A2<sup>HbQ India</sup> ( $\alpha\alpha^{\text{HbQ India}}\delta\delta$ ). Ideally variants detected while analyzing HbA1c should be further investigated for confirmation and result of which should be shared, discussed and the patient should be encouraged for screening of available family members for relevant variant hemoglobin. Combination of cation exchange HPLC and capillary electrophoresis in this case was sufficient to arrive at conclusion.

**Keywords:** HbA1c, Hb D Punjab, Hb Q India, Compound heterozygous, HPLC, Capillary electrophoresis

## INTRODUCTION

Hemoglobin a1c is fraction of hemoglobin that is formed from slow, irreversible non-enzymatic glycation at one or both N-terminal valines of the beta globin chain. The addition of glucose to hemoglobin is directly proportional to the blood glucose level and since the average life span of red cells is 120 days, HbA1c can be used as a tool to monitor glycemic control over the preceding 2 to 3 months.<sup>1</sup> Although HbA1c analysis by HPLC may give indication of presence of variant hemoglobin, protocol of reporting and management of the incidental detection of

hemoglobin variants is not well established. Many clinical laboratories do not report the presence of hemoglobin variants, whereas others report them only if they interfere with HbA1c measurement.<sup>2</sup>

Hemoglobinopathies consist of thalassemia and variant haemoglobin, a major health problem in the Indian subcontinent.<sup>3</sup> According to world health organization (WHO) 5% of the world population is a carrier for haemoglobin disorders.<sup>4</sup> Haemoglobin D (Hb D), a haemoglobin variant, occurs mainly in north-west India, Pakistan and Iran.<sup>5</sup>

After Hb S, Hb D could be considered as one of the commonly found abnormal hemoglobin in western part of India with quite a few reported cases. Heterozygous Hb D does not produce any clinical or hematological symptoms, but its association with other structural variants or thalassemia may not be clinically silent.<sup>5,6</sup>

Hb Q India may be clinically silent or may show mild degree of microcytic anaemia. The clinical and haematological picture may get complicated if it is found in association with other abnormal hemoglobinopathy or with thalassemia.<sup>7-9</sup>

## CASE REPORT

A 48 years old female belonging to Punjabi family was referred for routine investigations. Blood was collected in K2 EDTA (Becton, Dickinson and Company; NJ, US).

Hemogram was performed on BC 6800 plus (Mindray; Shenzhen, China) which showed mild microcytic hypochromic anaemia (Table 1).

**Table 1: Red cell parameters of the patient.**

Parameters	Value
Haemoglobin (g/dL)	10.2
RBC count (10 <sup>6</sup> /uL)	4.48
Haematocrit (%)	31.7
MCV (fL)	70.8
MCH (pg)	22.8
MCHC (g/dL)	32.2
RDW CV (%)	28.9
RDW-SD (fL)	74.7

HbA1c was analyzed on D10 (Bio-Rad laboratories; California, US) using faster HbA1c protocol. Upon reviewing chromatogram, unexpected unknown peak reflecting the presence of hemoglobin variant was found. Though HbA1c result was within normal limits and patient was non diabetic, HbA1c result was reanalyzed on c311 (Roche; Basel, Switzerland) using Tina-quant haemoglobin A1c assay.

For identification of variant, sample was analysed on D10 using HbA2/F/A1c protocol which revealed presence of 4 distinct peaks along with small peak of Hb A2 (Figure 1). These peaks were identified based on HPLC retention times depicted in the Bio-Rad library of abnormal haemoglobin.

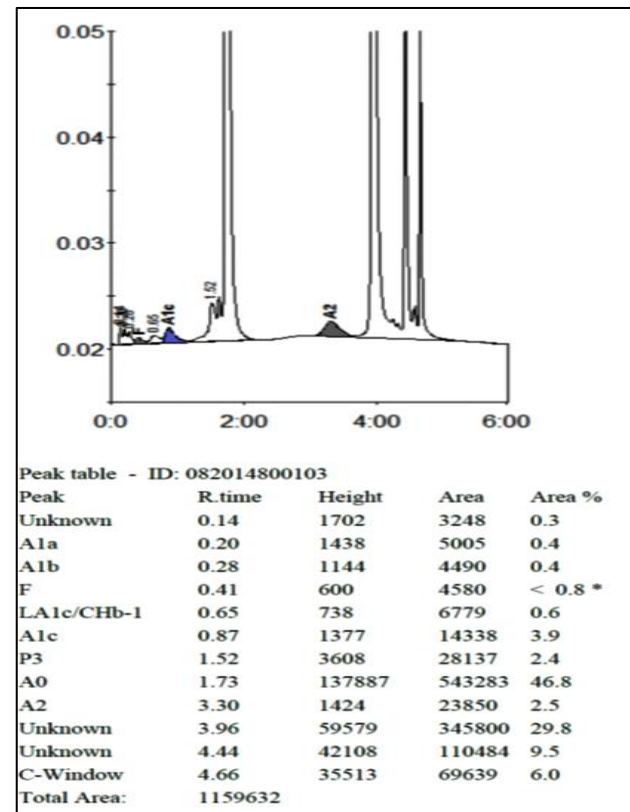
Sample was also analysed on variant V-II instrument (Bio-Rad laboratories; California, US) using beta thal short program, which showed similar results confirming presence of Hb D variant in D-window (Figure 2).

Solubility test for haemoglobin S using sodium dithionite as reducing agent was negative.

Usually Hb Q India ( $\alpha\alpha^{\text{HbQ India}}\beta\beta$ ) in its heterozygous form constitute 8-24% of total haemoglobin with Retention time of  $4.45 \pm 0.01$  minutes on Bio-Rad D10. In present case Hb Q India eluted at 4.45 minutes with 9.5% as unknown fraction. On Bio-Rad V-II it got eluted as unknown fraction of 10.8% with 4.69 minutes retention time.

Hb D Punjab ( $\alpha\alpha\beta\beta^{\text{HbD Punjab}}$ ) elutes around 3.9 minutes retention time on Bio-Rad D10 as an unknown fraction. In this case an unknown fraction was eluted at 3.96 minutes retention time with 29.8% concentration. On Bio-Rad VII, Hb D Punjab got eluted with 4.09 minutes retention time and 26.1% concentration as peak in dedicated D-Window.

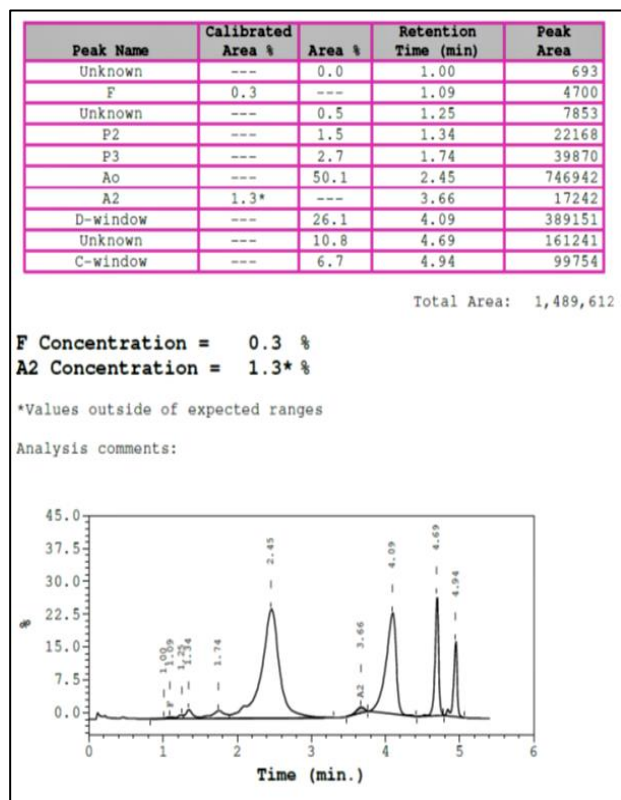
One additional fraction consisted of hybrid of Hb Q India/Hb D Punjab ( $\alpha\alpha^{\text{HbQ India}}\beta\beta^{\text{HbD Punjab}}$ ) got eluted as an unknown peak with concentration of 6% at 4.66 minutes retention time on Bio-Rad D10 and concentration of 6.7% at 4.94 min retention time on Bio-Rad variant V-II.



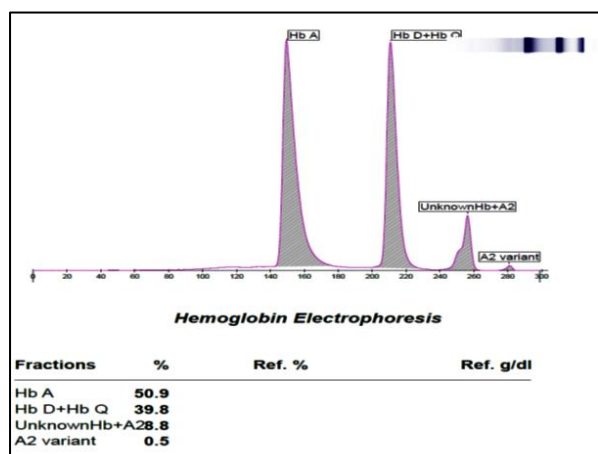
**Figure 1: Bio-Rad D-10 HPLC chromatogram of four peaks of Hb A, unknown 1 (Hb D Punjab), unknown 2 (Hb Q India) and C-window (Hybrid of Hb D Punjab/Hb Q India).**

While analysing sample on capillary electrophoresis (Sebia; Lisses, France), presence of four distinct peaks (Figure 3) were found as follows; A major peak in zone 9/Z(A) of Hb A constituting 50.9%. A second peak of 39.8% in zone 6/Z(D). Both variants; Hb D

Punjab and Hb Q India co-eluted in Hb D zone. Third peak of 8.8% in zone 3-4/Z(A2-C) showing merging of Hb A2 with an unknown variant. This unknown variant represented hybrid molecule of Hb D Punjab/Hb Q India which was also present as an unknown peak in Bio-Rad D10 and variant V-II with concentration in similar range. Last and a small fourth peak of 0.5% in zone 1 confirmed the presence of Hb A2 variant; A2<sup>HbQ India</sup> ( $\alpha\alpha^{\text{HbQ India}}\delta\delta$ ).



**Figure 2: Bio-Rad variant V-II HPLC chromatogram of four peaks of Hb A, D-window (Hb D Punjab), unknown (Hb Q India) and C-window (Hybrid of Hb D Punjab/Hb Q India).**



**Figure 3: Capillary electrophoresis pattern of four peaks of different concentration of Hb A, Hb D + Hb Q and unknown variant + Hb A2 and Hb A2 variant.**

Based on results of three different instruments, final diagnosis of compound heterozygous; Hb D Punjab/Hb Q India was made. Parental screening was advised for abnormal haemoglobin variants but unfortunately, they were not available.

## DISCUSSION

As HbA1c by HPLC is widely used not only for diagnosis and monitoring the treatment of diabetes, but also as a screening test in health checkup, and detection of Hb variant is an intrinsic part of assay, it is expected to detect many silent cases of hemoglobinopathy. Laboratories should have clear protocol to approach such cases and should report about presence of hemoglobin variant in the report.<sup>10</sup>

Many variants interfere with Hb A1C result by HPLC as haemoglobin variants or its glycated derivatives cannot be separated from Hb A or HbA1c. If variant haemoglobin happens to coelute with HbA1c, it will grossly overestimate HbA1c. If retention time of variant haemoglobin and Hb A is similar but retention time of glycated haemoglobin variant is different from HbA1c, then there will be underestimation of HbA1c.<sup>11</sup>

Accidental findings of silent carrier stage of variant haemoglobin should also be reported as this may provide direct health benefit to carriers and also play important role in reproductive decision making to modify risk for offspring of serious conditions.<sup>10</sup>

Haemoglobin Q-India is a structural variant caused by the mutation AAG→GAG (Asp→His) in the position of codon 64 of the alpha1 gene.<sup>7,12</sup> Hb Q India was first reported in Sindhi family in 1972 with beta thalassaemia.<sup>12</sup> Later on many cases have been reported and the prevalence of HbQ India is estimated to be 0.4% in India and is commonly found in Sindhi ethnicity.<sup>7</sup> Normally, HbQ India is clinically silent. As the haemoglobin 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.<sup>7,9</sup> Association with other beta chain variant or thalassaemia or iron deficiency may produce microcytic hypochromic anaemia.<sup>9,13</sup>

Haemoglobin D differs structurally from normal Haemoglobin A at 121<sup>st</sup> position on beta chain, where glutamic acid is replaced by glutamine ( $\beta^{121}$ ; GAA→CAA; Glu→Gln) and was first reported in 1951.<sup>5,14,15</sup> Haemoglobin D in its heterozygous form is not uncommon in North-West part of India; being approximately 2% among Sikhs in Punjab and 1% in Gujarat, but its association with other beta chain variants like Hb S or Hb E is rare.<sup>5</sup> Documented cases of its association with alpha chain variants like Hb Q India are fewer.<sup>16</sup>

## CONCLUSION

Many clinical laboratories do not elect to report variant hemoglobin, detected while measuring HbA1c, may be because of clinically silent nature of hemoglobin variant. Authors are of an opinion that not only variant should be reported but clinical laboratory should be prepared to discuss this with patients or clinician and urge them to investigate fully including relevant family screening to help them. Since the prevalence of Hb D Punjab and Hb Q India is not that uncommon in North West part of India, compound heterozygosity should also be not that uncommon as it was previously thought. Findings of unknown peaks in HPLC or capillary electrophoresis may cause diagnostic dilemma. When encountered, documentation of such unusual cases may help in diagnosis as well as persuading the family for screening of available family members for risk modification especially in antenatal condition. Though most of the time it is clinically silent, its combination with other prevalent variants such as Hb S, may aggravate clinical picture and by documenting as well as pre-marital screening in applicable individual, future occurrence of such combination can be prevented. In most of the cases combination of cation exchange HPLC and/or capillary electrophoresis with screening of available family members, is sufficient to arrive at conclusion. However, in few cases amplification refractory mutation system (ARMS) PCR is required which is useful tool for quick identification of any uncommon variant of globin genes for which the mutation is previously known. For definitive diagnosis or for variants where mutations are not known, DNA sequencing is required.

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## REFERENCES

- Nasir NM, Thevarajah M, Yean CY. Haemoglobin variants detected by hemoglobin A1c (HbA1c) analysis and the effects on HbA1c measurements. *Int J Diabetes Dev Ctries*. 2010;30(2):86-90.
- Behan KJ, Storey NM, Lee HK. Reporting variant hemoglobins discovered during hemoglobin A1c analysis- common practices in clinical laboratories. *Clin Chim Acta*. 2009;406:124-8.
- Dangi CBS, Kaur M, Banerjee N, Angastiniotis M, Eleftheriou A, Singh M et al. Burden of Haemoglobinopathies in Madhya Pradesh, Central India. *Int J Biotechnol*. 2013;111:224-42.
- WHO-Executive Board EB118/5. Prevalence of Haemoglobinopathies. 118<sup>th</sup> Session Report by the Secretariat on Thalassemia and other haemoglobinopathies. 2006;1-8.
- Desai D, Dhanani HJ, Shah M, Dayal N, Kapoor A, Yeluri S. Homozygous Hemoglobin D Disease: A Case Report. *Internet J Pathol*. 2003;3(1):1-4.
- Shanthala Devi AM, Rameshkumar K, Sitalakshmi S. Hb D: A Not So Rare Hemoglobinopathy. *Indian J Hematol Blood Transfus*. 2016;32(1):294-8.
- Desai DV, Dhanani HJ, Kapoor AK, Yeluri SV. HbQ-India in a Sindhi family: an uncommon hemoglobin variant. *Lab Hematol*. 2004;10(4):212-4.
- Panigrahi I, Bajaj J, Chatterjee T, Sakena R, Mahapatra M, Pati HP. Hb Q India: Is it always benign? *Am Hematol*. 2005;78:245-7.
- Desai D, Parmar C, Dhanani HJ, Patel RZ, Master DC. A Rare Case of Co-Existent Hb Q India-Beta Thalassemia Trait. *Internet J Hematol*. 2006;3(1):1-4.
- Lewis MR, Macauley RC, Sheehan PR, Staten MA, Phillips LS, Rasouli N et al. Management of Hemoglobin Variants Detected Incidentally in HbA1c Testing: A Common Problem Currently Lacking a Standard Approach. *Diabetes Care*. 2017;40(2):8-9.
- Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. *Clin Chem*. 2001;47:153-63.
- 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.
- Wiwantikit V. Secondary and tertiary structure aberration of alpha globin chain in haemoglobin Q-India disorder. *Indian J Pathol Microbiol*. 2006;49(4):491-4.
- Itano H. A third abnormal hemoglobin associated with hereditary hemolytic anaemia. *Proc Nat Acad Sci USA*. 1951;37:775-7.
- Baglioni C. Abnormal human hemoglobin. VII. Chemical studies on hemoglobin D. *Biochem Biophys Acta*. 1962;59:437-49.
- Colaco S, Surve R, Sawant P, Nadkarni A, Ghosh K, Colah R. HbD Punjab/HbQ India Compound Heterozygosity: An Unusual Association. *Mediterr J Hematol Infect Dis*. 2014;6(1):e2014072.

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