Case Report

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Pathology Section

Compound Heterozygosity for Haemoglobin D Punjab and Haemoglobin Q India: A Rare Case Report

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ABSTRACT

Haemoglobin variants constitute an important group of inherited disorders with variable clinical and haematological manifestations. The co-existence of structural variants involving both α and β globin chains is uncommon and may present diagnostic challenges during routine haemoglobinopathy screening. The authors hereby report a rare case of compound heterozygosity for haemoglobin D Punjab and haemoglobin Q India identified during haemoglobin variant analysis. A 19-year-old Sindhi unmarried woman was referred for haemoglobinopathy evaluation during investigation for menstrual irregularities. High-Performance Liquid Chromatography (HPLC) performed on the Bio-Rad D-10 system demonstrated a reduced Haemoglobin (HbA0) fraction (50.4 percent) with normal HbA2 (2 percent). A prominent peak of 25 percent at a retention time of 3.94 minutes suggestive of HbD Punjab, while an additional 13.7 percent peak at 4.66 minutes was suggestive of HbQ India. An intermediate 8.1 percent peak eluting in the HbC window was interpreted as a hybrid fraction produced by interaction between the two variants. Molecular confirmation using bidirectional Sanger sequencing identified heterozygous variants in both the β globin gene {Haemoglobin Subunit Beta (HBB) c.364G>C; p.Glu122Gln} and the α globin gene (HBA1 c.193G>C; p.Asp65His), confirming compound heterozygosity for HbD Punjab and HbQ India. The patient demonstrated mild microcytic anaemia without clinical features of haemolysis. Recognition of the characteristic HPLC pattern is important because the hybrid fraction may mimic HbC and lead to diagnostic confusion. Although this genotype is usually clinically benign, accurate identification is essential for genetic counselling and family screening.

Keywords: Haemoglobinopathy, High-performance liquid chromatography, Hybrid haemoglobin, Sanger sequencing, Structural haemoglobin variant

CASE REPORT

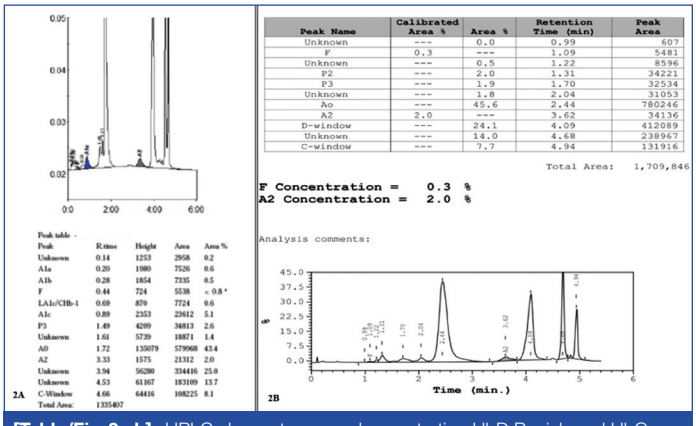
A 19-year-old Sindhi unmarried woman presented with menstrual irregularities and mild anaemia and had been receiving treatment for menstrual irregularities for five months. She was referred by the gynaecologist for haemoglobin variant analysis using HPLC as part of the diagnostic evaluation. There was no history of blood transfusion, jaundice, haemolytic episodes, or hospitalisation. Clinical examination revealed no hepatomegaly or splenomegaly. A detailed family history of haemoglobinopathies could not be obtained at the time of presentation. No clinical features suggestive of haemolytic anaemia were observed.

Diagnostic Assessment

Haemoglobin variant analysis was performed using HPLC on the Bio-Rad D-10 Haemoglobin Testing System. The chromatogram demonstrated a reduced HbA0 fraction (50.4%) with a normal HbA_2 level (2%). A prominent 25% peak at a retention time of 3.94 min eluted within the manufacturer-defined "unknown" window and was suggestive of HbD Punjab. An additional 13.7% peak at 4.66 min also eluted within the "unknown" window and was suggestive of HbQ India. A separate 8.1% peak was detected in the HbC window at 4.53 minutes and was interpreted as a hybrid fraction resulting from the co-inheritance of the two variants. The HPLC findings [Table/Fig-1] were suggestive of compound heterozygosity for HbD Punjab and HbQ India. Repeat analysis using the Bio-Rad Variant Haemoglobin Testing System demonstrated a concordant chromatographic profile [Table/Fig-2a,b].

Investigation	Observed value (%)	Retention time (min)	Biological reference interval
Foetal Haemoglobin (HbF)	0.8	0.44	0.0-2.0
Haemoglobin A_0 (Hb A_0) (Calculated)	50.4	1.72	94.3-98.5
Haemoglobin A_2 (HbA_2)	2.0	3.33	1.5-3.7
Haemoglobin D (HbD)	25	3.94	0–0
Haemoglobin C window (Hybrid)	8.1	4.53	0–0
Other (Unknown peak)	13.7	4.66	0–0

[Table/Fig-1]: Haemoglobin variant analysis on HPLC.



[Table/Fig-2a,b]: HPLC chromatograms demonstrating HbD Punjab and HbQ India variant patterns obtained using two Bio-Rad analysers: (a) Bio-Rad D-10 Haemoglobin Testing System; and (b) Bio-Rad VARIANT Haemoglobin Testing System.

Molecular Confirmation

Molecular characterisation by bidirectional Sanger sequencing identified two distinct heterozygous globin gene variants. Analysis of the β-globin gene (HBB) revealed a single nucleotide substitution, NM_000518.5:c.364G>C, resulting in the missense variant p.Glu122Gln, which is consistent with the haemoglobin D Punjab variant. Concurrent sequencing of the α-globin gene (HBA1) demonstrated a heterozygous nucleotide change, NM_000558.4:c.193G>C, predicting the amino acid substitution p.Asp65His, which is consistent with haemoglobin Q India. Variants

were annotated according to Human Genome Variation Society (HGVS) guidelines using RefSeq transcripts NM_000518.5 and NM_000558.4.

The electropherogram demonstrated dual peaks at the variant positions [Table/Fig-3a,b], confirming heterozygosity for both mutations. These results confirmed the molecular diagnosis of compound heterozygosity for HbD Punjab and HbQ India.

Additional Haematological Investigations

The complete blood count revealed mild anaemia with haemoglobin of 9.9 g/dL and reduced haematocrit (32.1%). The red blood cell count was 5.24 $\times 10^6$/µL. Red cell indices showed a microcytic, hypochromic pattern (MCV 61.3 fL, MCH 18.9 pg, MCHC 30.8 g/dL), accompanied by an elevated RDW (20.6%), indicating anisocytosis [Table/Fig-4]. Peripheral smear examination showed microcytic hypochromic red blood cells without target cells or basophilic stippling. The reticulocyte count was 1%. Iron studies were not performed at the time of evaluation.

Therapeutic Intervention and Recommendations

No specific therapeutic intervention was initiated for haemoglobinopathy, as the patient was clinically stable with mild anaemia. Ongoing treatment for menstrual irregularities was continued as advised by the treating clinician. The patient was counselled regarding the typical benign clinical course of compound heterozygosity for HbD Punjab and HbQ India. Family screening was recommended to identify potential carriers among first-degree relatives and facilitate appropriate genetic counselling. The patient did not return for follow-up after the initial evaluation. Therefore, the subsequent clinical course, completion of genetic counselling, and family screening could not be assessed.

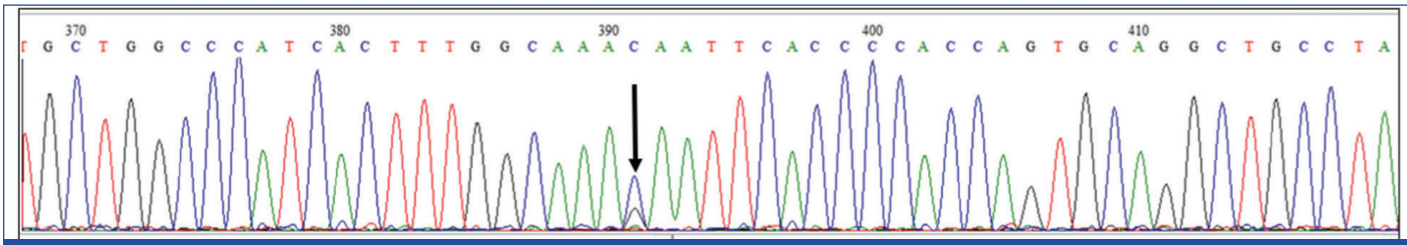
DISCUSSION

Haemoglobinopathies remain a significant global health concern, with 5 to 7 percent of the world population carrying a clinically relevant haemoglobin variant [1,2]. In the Indian population, β-thalassaemia trait is the most prevalent abnormality (carrier frequency ~3-4%), followed by sickle cell disorders and HbE [3,4].

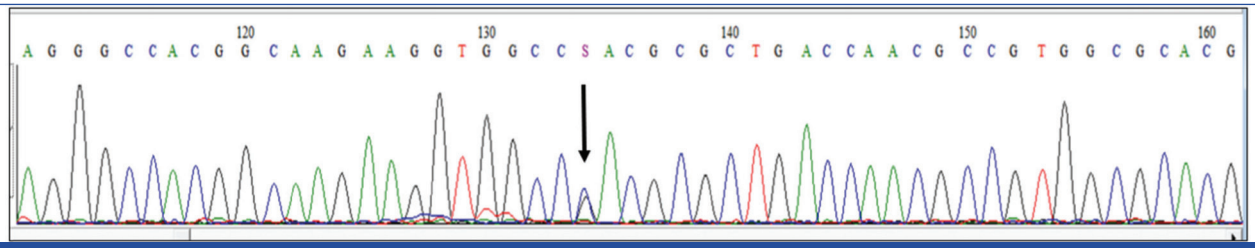
Compound heterozygosity involves the inheritance of two different globin gene variants. In the Indian population, it most often reflects β-thalassaemia in combination with sickle haemoglobin or HbE. Co-inheritance of structurally abnormal variants affecting both α and β globin genes is distinctly uncommon [5].

The HbD Punjab, also termed HbD Los Angeles, is a β globin structural variant caused by a missense mutation in HBB (c.364G>C), resulting in the substitution of p.Glu122Gln, classically described as β121 (GH4) Glu→Gln [6]. HbQ India is an α1 globin variant resulting from a mutation in HBA1 (c.193G>C), producing the substitution of p.Asp65His, historically denoted as α64(E13) Asp→His [7]. Compound heterozygosity for HbD Punjab and HbQ India is exceptionally rare, primarily because both variants have low allele frequencies in the population. Consequently, the likelihood of inheriting both variants in the same individual is exceedingly low, and only a few cases have been reported worldwide.

In previously published cases, HPLC has consistently demonstrated four principal peaks corresponding to HbA, HbD -Punjab, HbQ India, and a hybrid haemoglobin fraction eluting in the HbC window [Table/Fig-5] [5,8-11]. HbD Punjab generally comprises less than 40% of total haemoglobin, while HbQ India usually remains below 20%, with a smaller but distinct hybrid peak. The chromatographic findings in the present case are concordant with those of previous reports.



[Table/Fig-3a]: Sanger sequencing chromatogram showing a heterozygous HBB variant, NM_000518.5:c.364G>C (p.Glu122Gln; HbD Punjab). The arrow indicates the mutation site.



[Table/Fig-3b]: Sanger sequencing chromatogram showing the heterozygous HBA1 variant, NM_000558.4:c.193G>C (p.Asp65His; HbQ India). The arrow indicates the mutation site.

Parameters	Observed value	Unit	Reference interval
Haemoglobin (Hb)	9.9	g/dL	12.0-16.0
Red Blood Cell Count (RBC)	5.24	$\times 10^6$/µL	4.5-5.9
Haematocrit (PCV)	32.1	%	40-50
Mean Corpuscular Volume (MCV)	61.3	fL	80-96
Mean Corpuscular Haemoglobin (MCH)	18.9	pg	27-33
Mean Corpuscular Haemoglobin Concentration (MCHC)	30.8	g/dL	32-36
Red Cell Distribution Width (RDW-CV)	20.6	%	11.5-14.5
Total Leukocyte Count (TLC)	10.0	$\times 10^3$/µL	4.0-11.0
Platelet count	4.52	$\times 10^5$/µL	1.5-4.5

[Table/Fig-4]: Complete Blood Count (CBC).

Sr No.	Authors	Year	Age/ Sex	HbD % distribution	HbQ % distribution	Hybrid peak (Eluting as HbC)
1	Higgins T et al., [8]	2012	39/F	30.5	9.5	7.1
2	Mutreja D et al., [9] (Two cases)	2013	26/M;	33.4	10	7.7
			29/M	32.6	09	7.6
3	Colaco S et al., [10]	2014	29/F	31.5	9.4	7.4
4	Parab S et al., [11]	2014	--	30.7	11.2	8.2
5	Madabhushi SC et al., [5]	2015	43/F	24.6	13.9	9.1
6	Current case	2026	19/F	25	13.7	8.1

[Table/Fig-5]: Reported cases of HbD-HbQ compound heterozygosity with HPLC distribution [5,8-11].

Published reports indicate that HPLC remains the primary screening method because it permits reliable quantification of haemoglobin variant fractions in a single analytical run [12,13]. Although this variant combination exhibits a characteristic chromatographic pattern, it may be misinterpreted in routine practice because the hybrid fraction elutes in the HbC window. This hybrid fraction represents haemoglobin tetramers formed by the combination of HbQ India α-globin chains and HbD Punjab β-globin chains, resulting in a distinct peak within the HbC window on HPLC.

Furthermore, the chromatographic findings may overlap with those of other structural haemoglobin variants, including HbC, HbE, HbD-Iran, and other variants with similar retention times. Therefore, careful interpretation of retention times, peak proportions, and the overall chromatographic pattern, together with molecular confirmation in selected cases, is essential for establishing the correct diagnosis. Accurate recognition of this rare compound heterozygous state helps avoid diagnostic misclassification, prevents unnecessary investigations, and facilitates appropriate genetic counselling and family screening. HbD Punjab affects the β-globin chain and HbQ India affects the α-globin chain. As both are structural variants that do not impair globin synthesis, affected individuals are generally asymptomatic or exhibit only mild haematological abnormalities. Although most reported cases demonstrate minimal haematological abnormalities, mild microcytosis and/or mild anaemia have also been described in some patients. In contrast, the present patient demonstrated marked microcytosis, hypochromia, and anisocytosis. Since iron studies were not performed, coexisting iron deficiency cannot be excluded. HBB sequencing was performed and did not reveal a β-thalassaemia variant. Therefore, the observed haematological abnormalities should not be attributed solely to the HbD Punjab-HbQ India compound heterozygous state and should be interpreted with caution.

Limitation(s)

Iron studies were not performed; therefore, coexisting iron deficiency could not be excluded. Parental testing could not be performed, limiting confirmation of the inheritance pattern.

CONCLUSION(S)

Compound heterozygosity for HbD Punjab and HbQ India is an exceptionally rare haemoglobinopathy that produces a characteristic HPLC profile and is usually associated with minimal clinical impact. Accurate chromatographic interpretation, supported by molecular confirmation, is essential to prevent diagnostic misclassification and facilitate appropriate genetic counselling. The present report highlights the diagnostic value of correlating HPLC findings with molecular analysis in rare haemoglobinopathies, reinforcing the need for heightened vigilance in atypical chromatographic presentations.

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