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Case Report

Pathology Section

Homozygous HbD Punjab- A Case Report of Family Study

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ABSTRACT

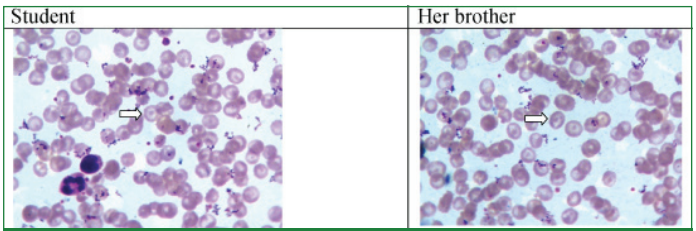
Homozygous HbD Punjab is a rarely reported haemoglobin variant. It co-elutes with other haemoglobin variants on alkaline and acid electrophoresis. However, on Cation Exchange-High Performance Liquid Chromatography (CE-HPLC), it elutes in the specific D-Window. Hence this is a sensitive method to clearly identify this haemoglobin variant. Here, a family study is presented in which both parents were HbD Punjab heterozygous and both children were HbD Punjab homozygous. All of them were clinically asymptomatic and were unaware of their status. This case is reported because of its rarity and clinical significance particularly if it occurs with other haemoglobin variants.

Keywords: HbS, High performance liquid chromatography, Microcytic, Thalassaemia

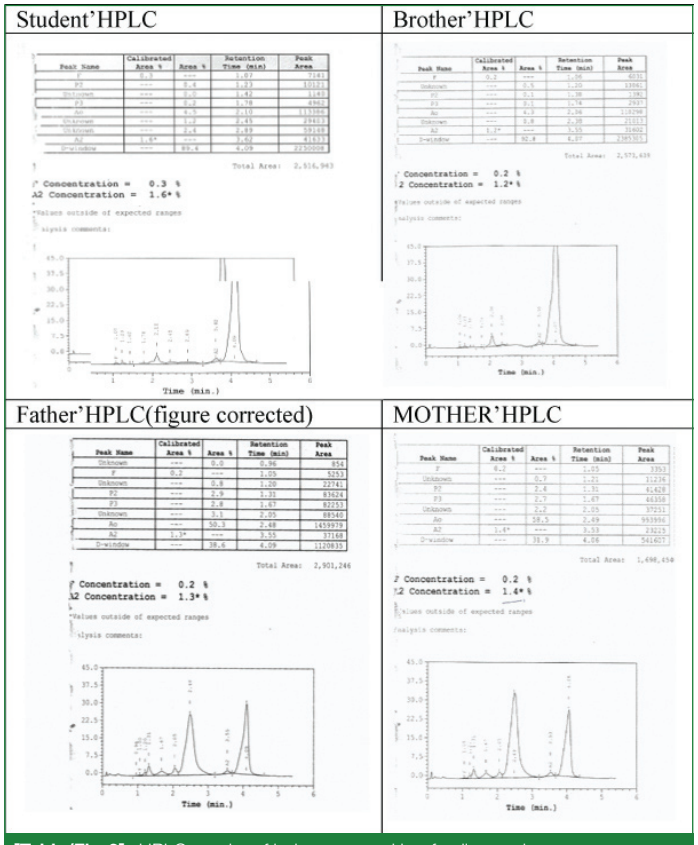
CASE REPORT

A 23-year-old female medical student came for the routine medical examination. The Complete Blood Count (CBC) revealed mild anaemia (haemoglobin 11.9 gm/dL, RBC count 5.26×10^6/µL, MCV 78.9 fL, MCH 27.5 pg, MCHC 34.9 g/dL). Peripheral smear revealed mildly microcytic red cells along with few target cells [Table/Fig-1]. These target cells are the red blood cells with a dark centre (haemoglobinized area) surrounded by a relative area of pallor and dark outer ring (containing haemoglobin). She does not have any clinical symptoms or hepatosplenomegaly. In the view of RBC count and red cell indices [Table/Fig-2], CE-HPLC was performed which showed a major band of haemoglobin eluting in D window comprising of 89.4% (retention time of 4.09 minutes) with HbA_0 4.5%, HbF 0.3% and HbA_2 1.6% [Table/Fig-2,3]. The chromatogram was suggestive of HbD Punjab homozygous. She was advised to get her family investigated for the suspected haemoglobinopathies. Her parents and her brother were called for clinical examination and haematological evaluation.

Complete blood counts of the brother revealed normal haemoglobin (15.3 g/dL) with mildly microcytic hypochromic indices [Table/Fig-2]. Red cells were predominantly normocytic normochromic with microcytic hypochromic along with few target cells [Table/Fig-3]. A major band in D window of 92.8% (retention time of 4.07 minutes) was seen on CE-HPLC with HbA_0 4.3%, HbF 0.2% and HbA_2 1.2%



[Table/Fig-1]: Peripheral smear of index case and her brother {arrow points towards target cell: red blood cells with a dark centre (haemoglobinized area) surrounded by a relative area of pallor and dark outer ring (containing haemoglobin)}.



[Table/Fig-3]: HPLC graphs of index case and her family members.

[Table/Fig-2,3]. The brother was also diagnosed as HbD Punjab homozygous on CE-HPLC.

The CBC parameter of mother revealed anaemia (Hb 8.6 g/dL) with microcytic hypochromic indices [Table/Fig-2]. Peripheral smear revealed microcytic hypochromic with few normocytic normochromic red cells. CE-HPLC showed D window of 31.9%

	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RBC COUNT (10^6/µL)	RDW-CV (%)	PCV (%)	HbD	HbA_0	HbF	HbA_2
Student	11.9	78.9	27.5	34.9	5.26	14.8	37	89.4%	4.5%	0.3%	1.6%
Brother	15.3	74.8	25.2	33.6	6.08	14.5	45.5	92.8%	4.3%	0.2%	1.2%
Mother	8.6	74.5	20.7	27.7	4.16	18.4	31	31.9%	58.5%	0.2%	1.4%
Father	15.7	89.5	28.9	32.3	5.43	13.8	48.6	38.6%	50.3%	0.2%	1.3%

[Table/Fig-2]: RBC parameters and CE- HPLC values of blood samples of the index case and her family members.

(retention time of 4.06 minutes), HbA_0 58.5%, HbF 0.2% and HbA_2 1.4% [Table/Fig-2,3]. The chromatogram was suggestive of HbD Punjab heterozygous.

Father's haemoglobin was normal (15.7 g/dL) with normocytic normochromic red blood cells seen in peripheral smear examination. RBC indices of father appear within normal range [Table/Fig-2]. CE-HPLC showed D window of 38.6% (retention time of 4.09 minutes), HbA_0 50.3%, HbF 0.2% and HbA_2 1.3% [Table/Fig-2,3], hence suggestive of HbD Punjab heterozygous.

The index case and her brother were HbD Punjab Homozygous and both the parents were HbD Punjab Heterozygous. None of them had any clinical symptom or hepatosplenomegaly and all of them were unaware of their HbD status.

DISCUSSION

About 7% of world population have mutation in genes encoding haemoglobin chains [1]. Genetic alterations in the globin part can affect the production rate of haemoglobin overall, or it can generate several haemoglobin variants by modifying the molecular structure of haemoglobin [1,2]. Hb D-Punjab is one of the variant which is derived from a point mutation in the beta-globin gene (HBB) in the first base of the 121 codon (GAA-CAA) with the substitution of glutamine for glutamic acid (Glu-Gln) [3].

Study done from India have shown Haemoglobin D-Punjab to occur mainly in Sikhs in Punjab (2%), followed by Gujarat population (1%) [4]. HbD have been found to occur as mainly heterozygous HbD trait, HbD thalassaemia, Hb S-D disease and the rare homozygous HbD disease. Homozygous HbD is usually seen to be associated with mild haemolytic anaemia and mild to moderate splenomegaly. Patients with coexistent Hb D and thalassaemia trait tend to have mild anaemia and are asymptomatic. Double heterozygosity of HbD with Hb S leads to anaemia which is moderately severe [5]. On electrophoresis, HbD coelutes with other haemoglobin variants [6]. CE-HPLC serves as a sensitive method for detection of HbD. The retention time of D window on CE- HPLC varies from 3.90 to 4.3 minutes [7]. HbD-Homozygous generally has HbD values between 70-90%. HbD Punjab heterozygous display a D-Window with variant percentage ranging from 33-39% [3]. The patient's peripheral smears generally show microcytic hypochromic red cells with few target cells [5].

The other differential for the diagnosis of homozygous HbD disease is HbD-beta thalassaemia. These two conditions should be differentiated by using the parameters like red cell indices, HbA_2 and HbF levels. The major concern for ruling out Hb D-beta zero thalassaemia is that homozygous HbD disease causes mild haemolytic anaemia, but co-inheritance of beta zero thalassaemia with it gives rise to chronic haemolytic anaemia of moderate severity [5]. In case of double heretozygous for HbD Punjab and beta zero thalassaemia, HPLC shows major band of HbD of 80-90% with mild elevation of HbF 3-6% and absent HbA_0 peak [8]. Hence, HPLC is helpful in differentiating both of these conditions.

The present study highlights a family study with children being HbD Punjab homozygous while parents being HbD Punjab heterozygous. Some studies done in the past have highlighted the prevalence of HbD Punjab. Pant L et al., studied 4800 cases and found haemoglobinopathies in 290 (6.04%) cases by CE-HPLC. Among these, 15 were HbD Punjab heterozygote (Hb D level 31-40% and near normal RBC parameters) and four cases which were double heterozygous of Hb D and beta thalassaemia trait (HbD level 77-81.3%, with raised HbA2 levels 3.8-6%). They did not come across any case of HbD Punjab Homozygous [9]. Sachdev R et al. studied 2600 cases and found hemoglobinopathies in 327 cases. Among them, 0.5% cases comprises of HbD-Punjab heterozygous. These were diagnosed on the basis of CE-HPLC. They did not came across any case of HbD Punjab Homozygous [10]. Srinivas U et al., found haemoglobinopathies in 0.55% cases in their study. Among these 0.55%, 7.8% (38 cases) showed D window. Among these, 23 were of heterozygous HbD Punjab, nine were homozygous HbD Punjab, two were HbS/D and four were HbD/β thalassaemia [11].

Patients with the coexistent HbD and thalassaemia trait can have mild anaemia and can be asymptomatic clinically, while those with co-existent HbD with HbS can present with moderately severe anaemia clinically [5].

CONCLUSION

HbD occurring in homozygous form is seen rarely and it usually presents with mild haemolytic anaemia and mild to moderate splenomegaly. Family study and counselling is needed especially to rule out the co-inheritance of HbD with other haemoglobin variants like HbS and beta-thalassaemia as they can give rise to clinical symptoms. CE-HPLC is a sensitive technique for making an accurate diagnosis of this entity.

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