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CORRESPONDENCE

Hb D: A Not So Rare Hemoglobinopathy

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Dear Editor,

Hb D is a β chain haemoglobin variant. It was first described by Itano in 1951 [1]. It differs in structure from Hb A at 121 position on β chain where glutamine replaces glutamic acid [2]. Hb D is known variously as Hb D Punjab, Hb D Los Angeles. It is the fourth most frequently occurring Hb variant [3].

Hb D Punjab occurs with greatest prevalence (2 %) in Sikhs of Punjab and in Gujarat (1 %). It is also found sporadically in blacks and Europeans, the latter usually seen in countries that have close association with India in the past [3].

HbD heterozygotes are clinically normal and homozygotes have a clinically mild phenotype. Hb D attains clinical significance in association with either β thalassemia or HbS.

We describe 12 patients, 9 from South Indian population (three cases of Hb D trait and six cases of Hb SD) and 3 from West Bengal (one case homozygous HbDD, one case of HbD β thalassemia and one case of HbD trait).

Blood samples collected in EDTA were subjected to complete blood counts, reticulocyte count and red cell indices on the Sysmex XT 1800i/XT 2000i/XT 4000i. Peripheral blood smears were prepared stained with Leishman's stain and examined. Sickling test was done where indicated by using 1 % sodium metabisulphite. G6PD screening was done using dye reduction test. The HPLC was done on BioRAD Variant II. The clinical data was retrieved from the medical records department.

HPLC was done on 1,460 cases over a period of 2½ years from September 2009 to February 2012. Five hundred and eighty-nine cases of haemoglobinopathies were detected. β Thalassemia trait was most common with 373 cases (63 %) being detected. β Thalassemia major was seen in 36 cases (6.1 %) and β thalassemia intermedia in 13 cases (2.2 %). Sickle cell disease was the next common with 28 cases of Sickle cell trait (4.7 %), 25 cases of sickle cell anemia (4.2 %) and 18 cases of sickle β thalassemia (3 %). HbE was seen significantly in the migrant population from Bengal with 24 cases of HbEE disease (4 %), 27 cases of HbE trait (4.5 %) and 14 cases of HbE β thalassemia (2.3 %). Twelve cases (2.0 %) showing Hb D were detected of which six cases were double heterozygous HbSD, one case of homozygous HbDD, one case of HbD β thalassemia and four cases of HbD trait. Eleven cases (1.8 %) of hereditary persistence of foetal haemoglobin (HPFH), four cases (0.6 %) of HPFH trait and four cases (0.6 %) of α thalassemia were also seen.

Hb D formed a significant percentage of the hemoglobinopathies (2 %). Most of them 9 of 12 cases were from South India and three cases from West Bengal. HbSD was seen in the South Indian population whereas homozygous HbDD and HbD β thalassemia seen in the migrant patients from West Bengal.

HbSD

Six cases of HbSD were reported, all from South India. Five of the cases had symptoms related to sickling. Three had hip joint pain, two of which required bilateral total hip

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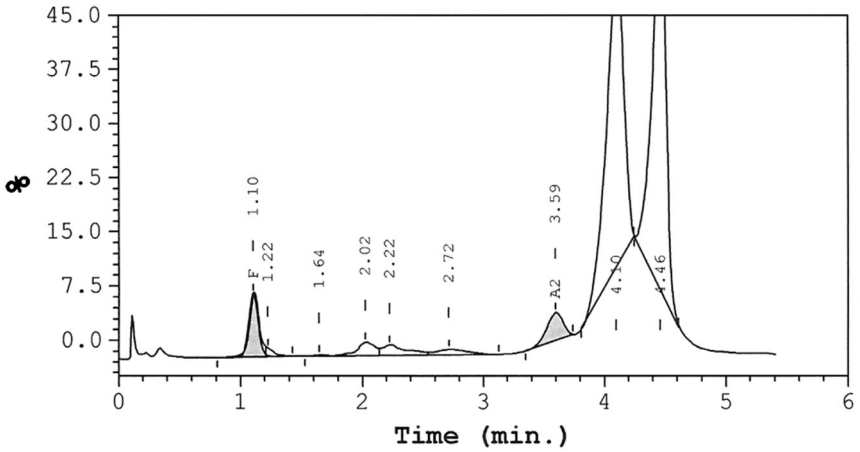
Fig. 1 HbSD

Peak Name	Calibrated Area %	Area %	Retention Time (min)
F	4.8*	---	1.10
Unknown	---	0.5	1.22
P3	---	0.1	1.64
Unknown	---	2.1	2.02
Ao	---	2.2	2.22
Unknown	---	1.5	2.72
A2	3.8*	---	3.59
D-window	---	42.6	4.10
S-window	---	42.5	4.46

F Concentration = 4.8* %
A2 Concentration = 3.8* %



replacement due to avascular necrosis and the third patient was diagnosed to have hip joint synovitis. Two patients (brothers) had repeated attacks of pain abdomen probably due to sickling crisis and episodes of jaundice. Three of the five patients required transfusions.

The youngest patient was a 18 month female who presented with fever, pallor, puffiness of face and hepatosplenomegaly. Sickling test was positive. Hb electrophoresis at alkaline pH showed Hb S of 90.5 %. The Hb electrophoresis of both parents showed Hb S around 40 %. The parents were diagnosed as sickle cell trait and the child as sickle cell anemia. However Sickling test was not carried out on the parents samples. The patient underwent HPLC at a later date which showed Hb F 4.8 %, HB S 42.5 % and Hb D 42.6 % and the diagnosis was revised as HbSD (Fig. 1).

All patients of HbSD had mild to moderate anemia (Hb range 7.0–11.4 g/dl) with MCV and MCH in normal range and high RDW indicating some degree of anisopoikilocytosis.

The HbD ranged from 27.1 to 43 %, HbS ranged from 20.8 to 44 % and HbF ranged from 3.2 to 25.3 %.

HbDD

A 18 year old female from West Bengal presented with recurrent episodes of jaundice requiring an occasional transfusion. She presented with mild microcytic hypochromic anemia and HPLC showed HbD of 83 % with normal Hb A_2 2.5 % (Fig. 2). HPLC done on her mothers sample showed HbD trait. A diagnosis of HbDD was made with suggestion to investigate the father to conclusively rule out HbD β thalassemia.

HbD β Thalassemia

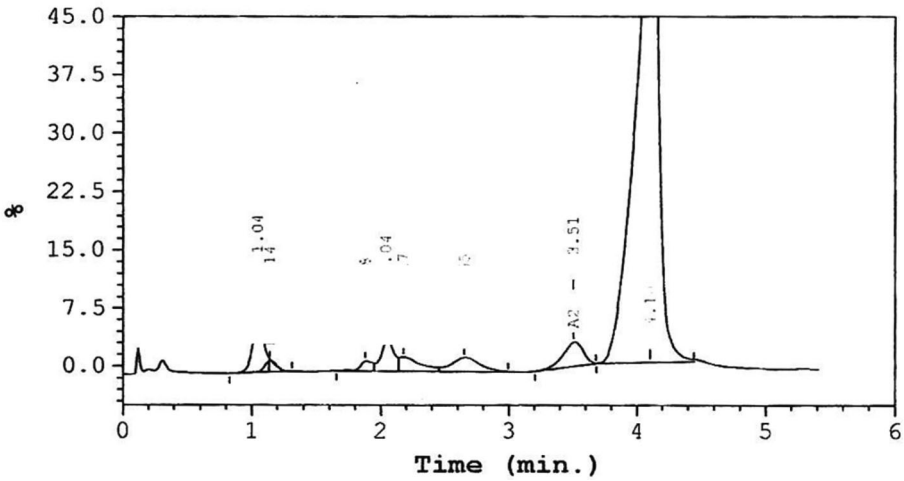
A 56 year old male from West Bengal presented with mild anemia requiring occasional transfusions, jaundice and hepatosplenomegaly. He was previously diagnosed as HbD. The peripheral smear showed microcytic hypochromic anemia. The HPLC done showed HbD of 88 % and HbA_2 of 4.3 % (Fig. 3). His wife was also investigated. Her CBC and HPLC were within normal limits. His son

Fig. 2 HbDD

Peak Name	Calibrated Area %	Area %	Retention Time (min)
Unknown	---	4.3	1.04
F	0.9	---	1.14
P3	---	0.9	1.88
Ao	---	2.8	2.04
Unknown	---	2.0	2.17
Unknown	---	2.7	2.65
A2	3.2	---	3.51
D-window	---	83.0	4.10

Total Area:

F Concentration = 0.9 %
A2 Concentration = 3.2 %



showed features of β thalassemia trait. Hence this patient was diagnosed as HbD β thalassemia in view of elevated HbA_2 and family studies.

HbD Trait

Four cases of HbD trait were detected three of which were parents of the above cases. The other case was a 1 year old male child who presented with anemia and hepatosplenomegaly requiring seven transfusions. His twin died with similar complaints. Peripheral blood smear showed haemolytic blood picture with presence of bite cells. However dye reduction test for G6PD was negative. HPLC showed HB A 72.2 %, Hb D 23.8 %, Hb F 1.8 % and Hb A2 1.5 %. Parents were evaluated to rule out HbD thalassemia. The father was normal and mother showed HbD of 36.8 %. The patient was diagnosed as HbD trait with concomitant enzyme deficiency to be ruled out. The other two patients were mother of the HbDD patient and father of the HbSD patient.

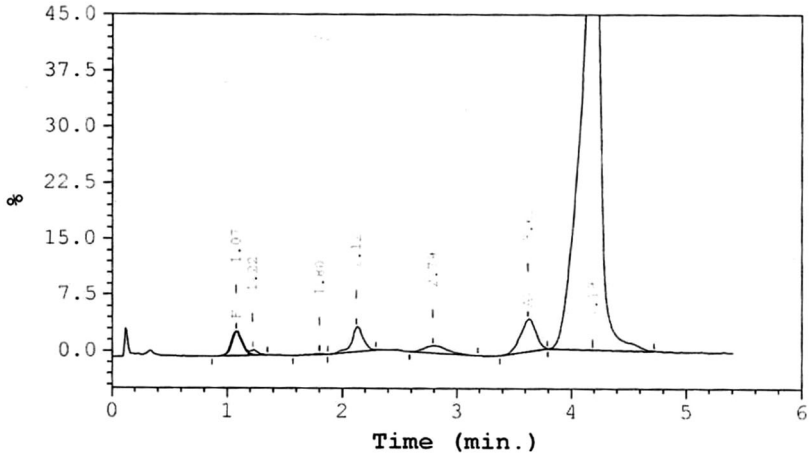
The haematological parameters and haemoglobin distribution by HPLC are summarised in Table 1.

HbD Punjab was first reported from Poona in a Sikh soldier from Hoshairpur district in East Punjab, who was apparently healthy [4]. HbD has hence been reported from Punjab and also from Gujarat. There are sporadic reports of HbD from other parts of the country including Bengal [5], Mysore [6], Madhya Pradesh [7], Uttar Pradesh [8] and Kerala [9]. HbD trait is clinically silent and is usually detected in screening programs for HbS. If the screening test done is alkaline gel electrophoresis and sickling test has not been done, it is likely to be mistaken for HbS as HbS and HbD move together in alkaline electrophoresis.

HbD Punjab also known as HbD Los Angeles is a β-chain variant and is characterized by a glutamic acid to glutamine substitution at codon 121 with electrophoretic mobility at alkaline pH is similar to HbS.

HbD has been described in both the heterozygous and homozygous states as well as in combination with HbS or β-thalassemia. Hb D disease (HbDD) is characterised by mild haemolytic anemia and mild to moderate splenomegaly. Both HbDD and HbD trait may not be clinically significant to seek medical attention. This mutation acquires clinical significance when it occurs in

Fig. 3 HbD β Thal



Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	2.5*	---	1.07	21936
Unknown	---	0.4	1.22	3493
P3	---	0.1	1.80	1187
Ao	---	2.7	2.12	24081
Unknown	---	1.6	2.79	14092
A2	4.3*	---	3.62	41023
D-window	---	88.2	4.19	792283

Total Area: 898,094*

F Concentration = 2.5* %
A2 Concentration = 4.3* %

Table 1 Hematological parameters and haemoglobin distribution by HPLC

Pt	Age/sex	Hb (g/dl)	MCV (fl)	MCH (pg)	RDW (%)	RBC (mil/µl)	HbA (%)	HbF (%)	HbA_2 (%)	HbD (%)	HbS (%)	Diagnosis
1	1.5/f	8.7	85	28	24	2.35	–	4.8	3.8	42.6	42.5	HbSD
2	28/f	9.5	94	35.3	14	2.69	2.3	25.3	1.9	43	25.4	HbSD
3	24/m	11.4	80.7	27.8	23.9	4.24	17.7	7.5	4.3	36.6	31	HbSD
4	1/m	9.2	102	33.1	24	2.48	72.2	1.8	1.5	23.8	–	HbDtrait
5 m/o 4	20/f	12.1	81.1	29.3	13	4.23	58.5	0.3	1.7	36.8	–	HbDtrait
6	33/f	7.0	83.7	27.1	25.1	2.5	3.3	3.2	3.8	40.7	44.2	HbSD
7 f/o 6	68/m	14.5	92.8	31.5	13.9	5.2	58.8	0.7	2.2	33.9	–	HbDtrait
8	24/f	8.8	93.8	30.3	18	2.9	36.4	11.7	2.7	27.1	20.8	HbSD
9	18/f	10	63.9	22.2	18.7	4.5	3.7	4.3	2.5	83.4	–	HbDD
10 m/o 9	38/f	10.6	77.5	25.4	18.1	4.18	57.7	1.1	1.8	35.9	–	HbDtrait
11b/o 3	28/m	11.5	74.9	28.3	18.2	4.07	2.8	10.3	3.3	42.6	36.8	HbSD
12	56/m	10.2	58.3	19.2	20.4	5.32	2.7	2.5	4.3	88.2	–	HbDβThal

combination with either β thalassemia or HbS [3]. This may explain occurrence of six cases of HbSD in our study. Compound heterozygosity for HbSD results in a mild to moderate haemolytic anemia and a clinical syndrome similar to that of sickle cell disease. HbD-β thalassemia is also generally a very mild condition. However, HbSD disease may manifest with variable clinical features.

HbF has an ameliorating effect on clinical phenotype in HbSS [10]. However similar effect is not seen in HbSD. Two of the more severe cases of HbSD had varying levels of HbF of 3.2 and 25.3 %. Two cases of mild HbSD had

HbF levels of 7.5 and 11.7 %. Similar findings have been reported in the Kuwaiti population [11].

The different HbD variants produce different severity of disease with HbS. HbD Punjab produces clinically significant condition like sickle cell disease, whereas HbD Iran and HbD Ibadan are noninteracting and produce benign conditions like Sickle cell trait [12, 13].

Homozygous HbDD disease is very rare with very few reported cases [9, 12]. HbDD disease presents with mild microcytic hypochromic anemia with or without mild splenomegaly. It is important to differentiate it from Hb D β thalassemia as it presents with chronic haemolytic anemia of moderate severity. Compound heterozygosity for HbD and β thalassemia produces a mild thalassemic condition with HPLC showing predominantly Hb D with Hb A_2 ranging from from high normal levels to levels similar to those seen in β thalassemia trait.

Hemoglobin D trait is clinically and haematologically silent as seen in three of our cases which were analysed as a part of family studies in cases of HbSD and HbDD. Combined hemoglobinopathy and enzyme deficiency have been reported [14]. Hb D trait can be symptomatic when associated with other causes for hemolysis like enzyme deficiency as suspected in one our cases.

Hb D formed 2 % of the cases in the present study, half of which were HbSD. Prior to diagnosis by HPLC, using alkaline gel electrophoresis a total of 254 cases of hereditary haemolytic anemias diagnosed over a period of 5½ years from 2003 to 2008 were detected. These include 54 cases (21 %) of thalassemia major, 91 cases (35.8 %) of thalassemia trait, 20 cases (7.8 %) of thalassemia intermedia, 26 cases (10 %) of sickle cell disease, 18 cases (7 %) of sickle cell anemia, 5 cases (1.9 %) of sickle cell trait, 29 cases (11.4 %) of sickle thalassemia, six cases (2.3 %) of HbE disease, three cases (1.1 %) of Hb E thalassemia disease, three cases (1.1 %) of HbE trait and one case HbD Punjab (0.3 %). The detection of HbD by HPLC is higher as cases earlier diagnosed as sickle cell anemia may be cases of HbSD as seen in one of cases.

HbD has been reported from Punjab (2 %) and also from Gujarat (1 %). There sporadic reports of HbD from other parts of India. The incidence of HbD may be underestimated as most of cases are reported from hospital based settings. As both HbD trait and HbDD disease are clinically and haematologically silent or mild, these cases do not seek medical attention and HbD comes to clinical attention when combined with HbS or β thalassemia. This may explain the high number of HbSD cases in our study. It is important to be aware of the existence of HbD in the South Indian population as the patients can mistakenly be labelled as sickle cell anemia if only alkaline gel electrophoresis is done as seen in one of our cases. Population based screening studies may give a more accurate estimate of the prevalence of HbD gene in the population.

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