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Original Research Article

Variants of Hemoglobin D Punjab - A Retrospective Study

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Abstract

Hemoglobin D Punjab or HbD Los Angeles is one of the commoner hemoglobinopathies. In β-chain there is substitution at position 121 i.e.121 Glu→Gln (Glutamic Acid is replaced by glutamine). The clinical spectrum of all the variants are usually ranges from asymptomatic state to mild hemolytic anemia and mild to moderate splenomegaly. Here, the retrospective study was carried out in general population of all the age groups in Dhiraj General Hospital, Vadodara, Gujarat. The duration of study was 8 months. Diagnosis of HbD Punjab was done by hemoglobin HPLC method with BIO-RAD D-10™ machine. Total 16 Hb D Punjab cases out of 1245 cases were found. Which were further categorized in Hemoglobin D trait (HbAD); Hemoglobin D disease (HbDD); Hemoglobin D-β–thalassemia (thalassemia trait/AD trait or DD homozygous) and Double heterozygous S and D (HbSD) entity. While HPLC reporting, it is very essential to keep these differentials of Hb D Punjab entity in suspected hemoglobinopathies because in today's scenario there has been change in geographic distribution of population. A collective data from clinical history, complete blood count, HPLC finding & sickling solubility tests enable definitive identification of hemoglobin D Punjab variant.

Keywords: Hemoglobin D Punjab, Hemoglobin D trait (HbA), Hemoglobin D disease (HbDD), Hemoglobin D-β–thalassemia (thalassemia trait/AD trait or DD homozygous), Double heterozygous S and D (HbSD).

INTRODUCTION

Hemoglobin D Punjab also known as HbD Los Angeles is one of the commoner hemoglobinopathies in India, Pakistan, England, Ireland, Holland, Turkey and Brazil. Its prevalence is 1-3% of population in north-west India especially in Punjab (Sikhs) and in Gujarat. In β-chain there is substitution at position 121 i.e.121 Glu→Gln(Glutamic Acid is replaced by glutamine). Hb D Punjab and Hb D Iran have same electrophoretic mobility but different retention times on HPLC [11].

Genotype of HbD can be detected by DNA amplification and globin chain analysis [1-3]. In high risk couples, prenatal diagnosis can also be used in detection of Hb D Punjab Disorders [4].

Hb D Punjab disorders have six forms [11]

- Hemoglobin D trait (HbAD);
- Hemoglobin D disease (HbDD);
- Hemoglobin D-β–thalassemia (thalassemia trait/AD trait or DD homozygous);
- Double heterozygous S and D (HbSD);
- Hemoglobin D Iran disease and
- Double heterozygous E and D (HbED).

The clinical spectrum of all the variants are usually ranges from asymptomatic state to mild hemolytic anemia and mild to moderate splenomegaly.

The carrier state (HbAD), homozygous (HbDD), HbD Iran Heterozygous and double heterozygosity for HbE & HbD are completely asymptomatic. Whereas, patients with compound heterozygous for HbD & β–thalassemia and HbD-S present with mild anemia; similar to β–thalassemia and moderately severe clinical presentation of sickle cell anemia respectively [5, 6].

MATERIALS AND METHODS

The retrospective study was carried out in general population of all the age groups in Dhiraj General Hospital, Vadodara, Gujarat. Duration of study was 8 months (August 2018- March 2019). Out of 1245 samples screened, 16 patients had hemoglobinopathy of HbD Punjab disorders (1.3 % prevalence). All cases showed clinical presentation ranging from relatively no symptoms to mild to moderate anemia and no palpable spleen to hepatosplenomegaly. Out of 16 cases; 2 cases of HbAD, 1 case of HbDD, 6 cases of HbD-βthalalesmia and 7 cases of HbSD were diagnosed.

Although there was neither HbDE nor Hb Iran was identified.

Diagnosis of HbD Punjab was done by hemoglobin HPLC (High Performance Liquid Chromatography) method with BIO-RAD D-10™ machine. The samples were collected from OPD and admitted patients in K3EDTA and transported to Central Laboratory with properly maintained temperature. The BIO-RAD D-10™ is an automated cat-ion exchange HPLC instrument that has been used to quantify HbA2, HbF, HbA along with screening hemoglobin variants like HbS, HbD, HbE and HbC in a single, highly reproducible system. This makes the D-10™ an excellent platform for screening hemoglobin variants and hemoglobinopathies along with thalassemia. College of American Pathologists studies have shown equivalence or superiority over electrophoretic methods (2003 CAP survey) [5, 6].

The machine operates on the principle of HPLC and the column comprises of a small cat-ion exchange cartridge, with requirement of 2 ml of blood sample (EDTA). The samples are injected into the analysis stream and separated by the cat-ion exchange cartridge using a phosphate ion gradient generated by mixing 2 buffers of different ionic strengths to elute the different hemoglobin. Changes in optical density at 415 nm are measured. A secondary filter at 690 nm corrects the effects caused by mixing buffers. The data is processed and the report giving the chromatogram where the different peaks are identified in defined windows with information like retention time, relative percentage and area [5, 6].

Blood samples were collected in 2 ml vacutainers containing EDTA as an anticoagulant. Complete blood count and red cell indices were measured by Beckman Coulter LH 750 hematology analyzer. Also observed peripheral smear findings (field stain), reticulocyte count, sickling solubility test (Sodium dithionate powder) and quantitative test for G6PD enzyme whenever required.

RESULT

Total 16 Hb D Punjab cases out of 1245 cases were found. The results are as follows.

Table-1: Age wise distribution of Hb D Punjab patients

Sr. no.	Hb D Punjab disorders	Prevalence (%)	Age			
			≤ 17 years	18 - 30 years	31- 40 years	≥ 41 years
1	Hb AD	12.5	1	-	1	-
2	Hb DD	6.25	1	-	-	-
3	Hb D- β thalalesmia	37.5	4	2	-	-
4	Hb DS	43.75	2	4	1	-

Out of 16 patients; 9 were males and 7 were female. Also incidental finding for Hb D Punjab was found in 2 cases, whereas rests of the cases were symptomatic.

Below are the laboratory details of each entity of Hb D Punjab Disorders (Table 2, 3).

Table-2: CBC finding of Hb AD

		gm/dl	%	fl	pg	%	M/ul	%			%
1	Hb AD	11	35.1	77.9	30.7	31.3	4	14.4	MCHC, Target cells	negative	2.5
2		10.3	33.7	84.4	33.8	30.5	3.8	13.9	MCHC	negative	2
3	HbD D	6.9	22.3	69.0	25.5	27.9	2.7	23.4	MCHC, Anisopoikilocytosis, target cells	negative	6
4	Hb D-β thal	11.4	36.6	90.3	30.5	28.4	4.8	13.9	MCHC, few target	negative	2.5
5		12	34.6	89.5	39.6	29.5	4.9	14.3	MCHC, few target	negative	2.5
6		11	33.2	83.5	32.9	27.5	5.5	12.3	MCHC, few target	negative	1
7		8.9	27.5	90.2	36.8	29.6	6.0	15	MCHC, few target	negative	2
8		11.0	32.9	84.8	29.8	28.2	4.1	14.8	MCHC, few target	negative	2.5
9		10.5	30.6	88.7	30.4	30.0	4.7	12.8	MCHC, few target	negative	2
10	Hb DS	9.3	27.6	80.1	26.2	27.3	2.9	18	MCHC, Sickle cells, target cells	Positive	3.5
11		6.5	20.4	67.8	28.9	28.8	3.2	20	MCHC	Positive	3
12		4.8	16.8	68.3	27.5	25.5	1.7	24	MCHC, Sickle cells, target cells	Positive	7.5
13		7.9	22.7	70.2	26.9	26.9	3.8	22	MCHC, Sickle cells, target cells	Positive	5
14		8.6	27	77.4	28.7	28.1	2.9	19	MCHC	Positive	8
15		9.2	29.1	74.5	29.4	29.0	3.7	19	MCHC	Positive	10
16		10.0	32	69.9	25.1	25.5	2.9	18	MCHC, target cells	Positive	6

(MCHC- microcytic hypochromic)

Table-3: Chromatogram findings of Hb AD

Patient			A0	A2	F	S window	unknown peak
1	Hb AD	R.Time(min.)	1.72	2.77	0.43	-	3.89
		Area %	49.2	0.9	<0.8	-	39.4
2		R.Time(min.)	1.88	2.56	0.48	-	3.94
		Area %	53.6	3.01	0.66	-	36.6
3	H	R.Time(min.)	1.88	3.08	0.77	-	3.87
		Area %	7.4	2.5	<0.3	-	88.9
4	Hb D-β thal	R.Time(min.)	1.76	2.89	0.44	-	3.89
		Area %	5.6	3.6	1.9	-	79.5
5		R.Time(min.)	1.71	2.66	0.48	-	3.78
		Area %	7.1	4.7	7.4	-	71.9
6		R.Time(min.)	1.86	2.77	0.43	-	3.87
		Area %	6.6	3.7	2.0	-	75.5
7		R.Time(min.)	1.51	2.86	0.41	-	3.88
		Area %	10.1	4.5	8.4	-	66.9
8		R.Time(min.)	1.66	2.87	0.49	-	3.89
		Area %	8.6	4.5	7.0	-	65.5
9		R.Time(min.)	1.76	2.89	0.44	-	3.89
		Area %	5.6	3.6	1.9	-	79.5
10	Hb DS	R.Time(min.)	1.74	3.10	0.49	4.12	3.87
		Area %	5.3	2.2	13.1	31.4	43.8
11		R.Time(min.)	1.69	3.11	0.42	4.12	3.85
		Area %	41.9	3.9	<0.8	23.4	27.8
12		R.Time(min.)	1.71	3.11	0.42	4.13	3.86
		Area %	28.2	3.7	<0.8	26.6	32.9
13		R.Time(min.)	1.77	3.07	0.55	4.25	3.88
		Area %	3.5	2.5	17.8	27.8	42.8
14		R.Time(min.)	1.74	3.02	0.51	4.15	3.89
		Area %	3.8	2.7	10.2	34.0	43.8
15		R.Time(min.)	1.69	3.01	0.48	4.92	3.89
		Area %	43.9	2.8	<0.8	29.4	24.8
16		R.Time(min.)	1.64	3.13	0.79	4.72	3.87
		Area %	6.3	2.5	16.1	35.4	48.8

The positive PBS findings are shown in Figure 1. (Microcytic Hypochromic RBC, Target cells, sickle cell)

FIGURE WITH LEGENDS

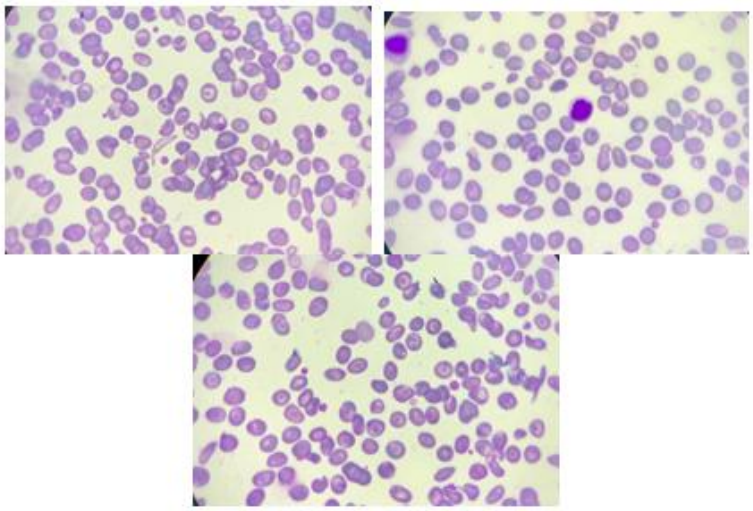
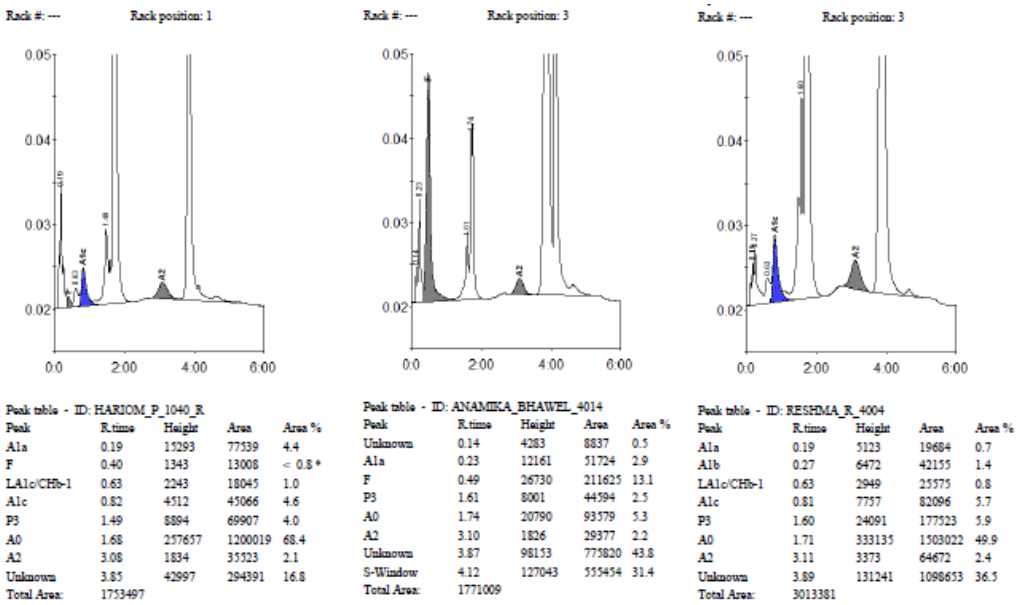


Fig-1: PBS showing Microcytic Hypochromic RBCs, Sickle cell and Target cells (40x)



Peak table - ID: HARIOM_P_1040_R

Peak	R.time	Height	Area	Area %
A1a	0.19	15293	77539	4.4
F	0.40	1343	13008	< 0.8 *
LA1c/CHb-1	0.63	2243	18045	1.0
A1c	0.82	4512	45066	4.6
P3	1.49	8894	69907	4.0
A0	1.68	257657	1200019	68.4
A2	3.08	1834	35523	2.1
Unknown	3.85	42997	294391	16.8
Total Area:			1753497	

Peak table - ID: ANAMIKA_BHAWEL_4014

Peak	R.time	Height	Area	Area %
Unknown	0.14	4283	8837	0.5
A1a	0.23	12161	51724	2.9
F	0.49	26730	211625	13.1
P3	1.61	8001	44594	2.5
A0	1.74	20790	93579	5.3
A2	3.10	1826	29377	2.2
Unknown	3.87	98153	775820	43.8
S-Window	4.12	127043	555454	31.4
Total Area:			1771009	

Peak table - ID: RESHMA_R_4004

Peak	R.time	Height	Area	Area %
A1a	0.19	5123	19684	0.7
A1b	0.27	6472	42155	1.4
LA1c/CHb-1	0.63	2949	25575	0.8
A1c	0.81	7757	82096	5.7
P3	1.60	24091	177523	5.9
A0	1.71	333135	1503022	49.9
A2	3.11	3373	64672	2.4
Unknown	3.89	131241	1098653	36.5
Total Area:			3013381	

Fig-2: HPLC reports of heterozygous state of Hb D Punjab

Clinical presentation of each subjects varied considerably. Out of 2 HbAD cases, one was found positive on accidental finding on routine checkup and both were clinically asymptomatic.

One individual with Hb DD had palpable spleen (+) with no significant chronic complaint. Total 6 cases were of HbD-β thalalesmia; of which 4 were pediatric patient with common clinical presentation of signs of anemia, weakness and tiredness and also repeatedly getting sick with either upper respiratory tract infection or fever. Rest of two was in adult age group with mild anemic signs.

Out of 7 individual with HbDS, 2 were pediatric patients, 5 were young adults and one of them was found positive accidentally during pre-donation formality in blood bank. Clinically every individual had signs of anemia (severity varied from mild to moderate). Also some had mild hepatosplenomegaly. Three individual had occasional pain in bone and abdomen as chief complaint.

DISCUSSION

Phenotype of carrier state (HB D Punjab Heterozygous) is completely asymptomatic. Prevalence of which is 3% in general north Indian population.[5,6] It is forth common hemoglobin variant found in the world and commonly found in north west India, Iran and Pakistan. It doesn't cause any abnormal pathophysiology in the heterozygous state. The individual is normal with almost normal RBC indices. On D-10™ Hemoglobin testing system, Hb D Punjab elutes in an unknown window at a retention time of approximately 3.8± 0.1 min., clearly demarcated from the HbS peak. The abnormal hemoglobin constitutes usually between 30-45% of the total Hb. HbF levels are normal, however the HbA2 values are on the lower side with range of 0.9-2.5%[8].

The homozygous state demonstrates mild hemolytic anemia. Hb D Homozygous is rare as compared to Hb D- β thalalesmia. On D-10™ Hemoglobin testing system, Hb D Punjab elutes in unknown window at a retention time of approximately 3.8± 0.1 min., clearly demarcated from the HbS peak. The abnormal Hb is >90%.Hb F and A2 are normal [7].

Similar to β thalalesmia trait, Hb D- β thalalesmia represent as asymptomatic with mild anemia. They may be β^0 β^0 or β^0 β^+. Patient may be asymptomatic with non-palpable spleen and have mild to moderate hemolytic anemia depending on β thalalesmia mutation. β^0 β^0 syndrome may exhibit symptomatic anemia with splenomegaly and may have a moderately severe clinical disorder. β^0 β^+ behavior is similar to β thalalesmia trait. Laboratory findings show microcytic hypochromic anemia and raised RBC count. Chromogram shows a major peak of HbD (70-90%) as unknown peak at retention time of approximately 3.8± 0.1 min., with elevated peak of HbF (3-20%). HbA0 peak is reduced and HbA2 values are elevated. Family studies are important to confirm diagnosis.

Double heterozygosity for HbD and HbS results in moderately severe clinical presentation of Sicke cell anemia. Here one gene carries the HbD mutation and the other gene carries HbS mutation- β^0 β^s. It is uncommon condition encountered in Punjabis and a higher frequency in Muslims in consanguineous marriages [9, 10].

CONCLUSION

In current scenario of community migration, consanguineous marriages, racial intermixing and changing population demographics; hemoglobin D Punjab disorders should no longer be considered as an

entity confined to Punjab region or in south Asian region. Thus, while reporting HPLC, it is very essential to keep these differentials of Hb D Punjab entity in suspected hemoglobinopathies. Also it is important to check for family history to facilitate error free counseling and management. A collective data from clinical history, complete blood count, HPLC finding & sickling solubility tests enable definitive identification of hemoglobin D Punjab variant.

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COMPETING INTERESTS

I declare that I have no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

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