

Detection of Hemoglobin D in Tharu Community of Banke, Nepal

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ABSTRACT

Background: Patients presenting with microcytic anemia, hepatosplenomegaly, abnormal hemoglobin level in electrophoresis and negative sickling test should have high suspicion for HbD disease. This occurrence is more in certain communities. While investigating the etiopathogenesis of subjects with microcytic anemia (n=30), we came across 3 patients with HbD disease. **Aim:** Present study describes the clinical and hematological findings of 3 patients. **Material and Methods:** This was an observational study conducted between August, 2014 to July, 2015 in Department of Pathology of Nepalgunj Medical College. Of 30 patients diagnosed as microcytic anemia, 3 patients were selected. **Results:** The patients belonged to Tharu Chaudhary community of Banke district of Nepal. Age of the patients ranged from 8 to 13 years. All the patients had generalized pallor. Two of 3 patients had fever, joint pains and jaundice. One of the patients had hepatosplenomegaly. Total hemoglobin ranged from 7.1 to 8.4 gm/dl. Patients had microcytic anemia. Sickling test was negative in all the 3 patients. Hemoglobin (Hb) electrophoresis revealed peaks in HbS region. Due to negativity of sickling test, the abnormal hemoglobin peak was interpreted as HbD. Thus, 2 patients had homozygous HbD disease while another patient had heterozygous HbD trait.

Key words: Anemia, HbD disease, microcytic.

INTRODUCTION

Patients with anemia and abnormal Hb level are occasionally seen by us at Nepalgunj Medical College teaching hospital. These patients belong to Tharu community of Banke district. In this region, vivax malaria is also endemic. Plasmodium infection might have influenced the evolution of abnormal hemoglobins in mid-western region of Nepal.

HbD Punjab is most commonly found in India. It is identical to HbD Los Angeles¹. It has also been found in Africa and Northern Europe¹. Four forms of HbD disease may exist. First, heterozygous HbD trait, where HbD is about 30 to 40%. This condition may be clinically silent. Second, homozygous HbD disease, where HbD is about 80% to 90%, but most of homozygous HbD patients have normal red cell indices, no evidence of hemolysis and have normal HbF and HbA₂ levels. Third, HbD thalassemia may cause mild hemolytic anemia, thalassemic red cell indices, no evidence of hemolysis and have normal HbF and HbA₂ levels. Fourth, hemoglobin SD disease which causes moderate to severe sickling disorder².

Herewith, we describe clinical and hematological features of 3 cases of HbD disease from this area.

MATERIAL AND METHODS

This study was conducted at Nepalgunj Medical College, Nepalgunj from August, 2014 to July, 2015. Thirty subjects with microcytic anemia were selected for establishing their etiology. The subjects were prospectively recruited and preformed proforma was filled up. Subjects were collected from neighbouring areas, e.g. Banke, Bardiya and Dang. Detailed investigations revealed a diagnosis of HbD disease in 3 of 30 subjects. These 3 subjects were labeled as **patients**.

Present study was done on 3 patients with microcytic anemia. About 2.5 ml of venous blood was collected from each patient in EDTA vial. Blood smears were also prepared and stained by Leishman method. Hemoglobin was estimated by cyanhemoglobin method. Hemogram was estimated using Nihon Kohden Celltac E, Europe GmbH, Rosbach Germany, a 5 parts differential coulter counter. It worked using the principle of volumetric impedance. Results of tests were obtained as hemoglobin (Hb), hematocrit (Hct), total red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW).

Preparation of hemolysate

Two milliliter of EDTA blood was washed thrice with normal saline. The packed cells were lysed with distilled water and were centrifuged. Supernatant was collected as hemolysate and stored at +4°C.

Agarose gel electrophoresis at alkaline pH 8.6 Hemolysate (5 µl) was transferred into well plates using unit applicator, the sample was applied into the alkaline agarose gel along with suitable controls and immediately placed in electrophoresis chamber. The chamber was connected to a power supply and

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electrophoresed for 45 min at 100V. This was used to separate and identify different hemoglobins by their migration patterns within an electric field. This agarose gel was then transferred to a developer unit where it was fixed, stained, de-stained and dried for 50 min. Later, agarose gel film was studied in computerized software (Biotec-Fischer GMBH 101, Germany).

RESULTS

Table I shows results of clinical findings of 3 patients with anemia. Age of patients ranged from 8 to 13 (median 9) years. All the patients had generalized pallor. Two patients had fever, joint pains and jaundice. Moderate hepatosplenomegaly was also detected in a patient (no.2).

Table II shows results of red cell indices in patients. Total hemoglobin ranged between 7.1 to 8.4 gm/dl. Total RBC was relatively higher in patient no.3 as compared to RBC counts in two other patients.

Patient no.	Age Yrs.	Pallor	Fever	Joint Pain	Jaundice	Liver	Spleen
1	13	+	+	+	+	np	np
2	8	+	+	+	+	4 cm	9 cm
3	9	+	-	-	-	np	np

np = non-palpable

Table I : Shows clinical findings in patients with HbD disease.

Patient no.	Hb gm/dl	Hct cc%	RBC million/mm ³	MCV fl	MCH pg	MCHC cc%	RDW %	Reticulocyte %
1	7.9	24.3	3.36	72.3	23.5	32.5	17.9	2
2	7.1	24.2	3.19	75.9	22.3	29.3	19.6	1.5
3	8.4	24	5.2	74	23	32	14	1.1

Table II : Results of red cell indices in 3 patients with HbD disease.

Patient no.	Sickling test +/-	Hemoglobin electrophoresis				Globin genotype	Globin phenotype	Clinical diagnosis
		HbA2 %	HbD %	HbF %	HbA %			
1	-	11.3	88.7	-	-	$\alpha_2 \beta^D / \beta^D$	$\alpha_2 \beta^D \uparrow \uparrow \uparrow$	Homozygous HbD disease
2	-	1	99	-	-	$\alpha_2 \beta^D / \beta^D$	$\alpha_2 \beta^D \uparrow \uparrow \uparrow$	Homozygous HbD disease
3	-	0.7	5.3	-	94	$\alpha_2 \beta \beta^D$	$\alpha_2 \beta \uparrow \uparrow \beta^D \uparrow$	Heterozygous HbAD disease (HbD trait)

+ positive, - negative

Table III : Results of sickling test and hemoglobin electrophoresis in patients with HbD disease

Table III shows results of sickling tests and Hb electrophoresis. Sickling tests were negative in all the patients. However, separate peaks were seen in HbS region at alkaline pH. Peaks in HbS region were interpreted as that of HbD due to negative sickling tests. Two patients had high level of HbD (88.7% and 99%), suggesting homozygous HbD disease. Another patient (patient no.3) had low level of HbD concentration (5.3%). Therefore, in this patient, a diagnosis of heterozygous HbD disease (HbD trait) was made. In this patient, HbA concentration was 94%.

DISCUSSION

Most important feature of this study was the detection of peaks in the region of HbS. Due to its failure to produce sickling, these peaks were interpreted as that of HbD and these patients were diagnosed as suffering from HbD disease (HbS and HbD are known to have similar mobility at alkaline pH. However, they can be separated by agar gel electrophoresis at an acid pH).

Two of 3 patients had high percentage of HbD (88.7% and 99%). In these 2 patients, a diagnosis of homozygous HbD disease (HbDD) was made. Another patient (patient no.3) had low level of HbD (5.3%). In this patient, a diagnosis of heterozygous HbD disease (HbAD trait) was made.

HbD disease is caused by β chain mutation at position 121, where glutamine replaces glutamic acid². Homozygous HbD disease (HbDD) is characterized by a mild microcytic anemia, poikilocytosis, minimal hemolysis and mild to moderate splenomegaly³. Osmotic fragility may be decreased¹.

All the patients had moderate anemia. In addition, one of the patients with homozygous HbD disease (no.2) had moderate hepatosplenomegaly. Two patients with homozygous HbD disease had jaundice. Jaundice might have developed as the result of excessive hemolysis, leading to rise in unconjugated bilirubin.

The patient with heterozygous disease (no.3) had relatively milder disease as compared to other 2 patients with homozygous HbD disease. High level of HbA in this patient might have resulted in a milder disease. Heterozygous HbD disease may be clinically silent^{4,5}. However, severe hemolysis developed in an HbD trait mother following physiological stress during twin pregnancy⁶. In addition, clinical variation in the behavior of HbD traits may occur. In a previous study⁵, 6 of 30 patients with HbD traits were symptomatic. A rare case of HbD trait has been described in a 1 year old child having jaundice, hepatosplenomegaly and hemolytic anemia⁷.

CONCLUSION

HbD disease is an uncommon condition present in Tharu community of Midwestern Nepal. Two of the 3 patients had rare homozygous HbD disease while another patient had heterozygous HbD disease

Conflict of Interest: None

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