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Case Report

High Performance Liquid Chromatography (HPLC): An Essential Tool for Diagnosis of Compound Heterozygous Form (HbSD) of Hemoglobin-D (HbD) with Hemoglobin-S (HbS)

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ABSTRACT

Hemoglobinopathies are group of disorders that are inherited resulting in increase morbidity and mortality among the affected individuals. Many deaths have been reported because of these disorders. Compound heterozygous form of Hemoglobin-D **(**HbD) with Sickle Cell Disease (SCD) is very rarely found in Indians. Here, we present the clinical and laboratory findings of such a rare case. Blood samples from a 43-year-old Muslim woman were taken and blood investigations including High performance liquid chromatography (HPLC), Complete Blood Count (CBC), Peripheral Blood Smear (PBS) were done. CBC showed presence of anemia with Hemoglobin (Hb) of 3.9 gm/dl which showed moderate hypochromic picture with reduced Red Blood Cell (RBC) mass. HPLC was performed on BioRad-D10 machine and two peaks were obtained; one for HbD and another for Hemoglobin-S (HbS). This showed the presence of compound heterozygous for HbS-D. Compound heterozygous form of HbD with HbS is a rare disorder. The clinical manifestations occurring in such patients can be serious; so early detection through HPLC of the patients having anemia is necessary for prompt management. Also genetic counseling should be advised in the patients presenting with such anemia.

Keywords: Compound heterozygous HbD-HbS, HPLC

INTRODUCTION

The groups of hematological illnesses that manifest clinically as an altered haemoglobin structure or inadequate haemoglobin production due to genetic abnormalities are called 'Hemoglobinopathies'.

The amino acid sequence of either the alpha or beta globin chains may be altered. This alteration is due to deletion, insertion, or substitution, resulting in hemoglobinopathies which may result into formation of flawed haemoglobin.

The HbS hemoglobinopathies was first discovered by Pauling L, et al. HbS is produced by change in gene sequence at 6th position from glutamic acid to valine.[1]

A hemoglobin variant on HPLC known as hemoglobin 'HbD-Punjab' is primarily found in Iran, Pakistan, and northwest India.[2] At 121 position, there is substitution of glutamic acid by glutamine resulting in formation of new hemoglobin variant Hemoglobin-D or HbD-Punjab.[3]

HbD-Punjab disorder has six different forms:[3]

- Hemoglobin D trait (Hb-AD);
- Hemoglobin D disease (Hb-DD);
- Hemoglobin D-Beta thalassemia (thalassaemia trait/ AD trait or DD homozygous);
- Double heterozygous D and S (Hb-SD);
- Hemoglobin D Iran disease;
- Double heterozygous E and S (Hb-ES).

CASE REPORT

A 43-year-old Muslim woman from a low socioeconomic status, born out of consanguineous marriage from Gandhidham reported having generalized weakness and decreased appetite for the past 15 to 20 days. She also had burning micturition due to Urinary tract infection (UTI). She was menopausal since past one year. For the past two years, she had experienced comparable symptoms for four to five times for which she was treated for anemia by her general practitioner. During the previous two years, she had numerous blood transfusions with iron chelation without experiencing any adverse reactions. On systemic evaluation she was conscious, oriented without any organomegaly. Results of blood investigations at our lab set-up were as shown in Table no. 1:

Table1: Relevant Hematological Parameters of Patients' Investigations

Hematological parameters	Results	Units	Reference Range
Haemoglobin	3.9	g/dl	12.5-16
WBCcount (TLC)	10360	cumm	4000-10500
Platelets	483000	cumm	150000-450000
MCV	74	fl	78-100
MCH	26.7	pg	27-31
MCHC	36.1	g/dl	32-36
RDW	19.4	%	11.5-14
LDH	231	U/L	100-190
Iron	53	ug/dl	50-160
VitaminB12	620	pg/dl	159-1000
Sodium	146	mmol/L	135-145
Potassium	4.6	mmol/L	3.5-5.1
Chloride	127	mmol/L	98-107

On the basis of above findings and on peripheral blood smear (PBS) (Figure1), diagnosis of hemolytic anemia was offered. Studied smear showed moderate anisiopoikilocytosis. Red blood cells (RBCs) showed predominantly microcytic picture with moderate hypochromia having reduced RBC mass. Normocytic RBCs were seen at places. Many sickled RBCs were seen with many fragmented RBCs (schistocytes). Occasional elliptocytes and tear drop cells were seen. All these findings were suggestive of Severe Hemolytic Anemia.

Sickling solubility (tube) test was positive in our case. Refer Figure 2: Right sided normal control blood sample is Negative. Left sided image shows tube with patient's sample which is Sickling Solubility test positive. It is positive due to cloudiness and loss of demarcated lines in the background paper. Increased reticulocyte count of 7.5% noted. Hemoglobin electrophoresis was not done in our case due to lack of set-up and directly HPLC was advised for detection of HB variants.

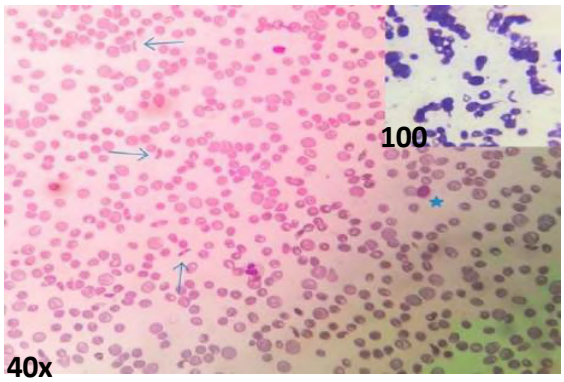

Figure 1: Microphotograph-Sickled RBCs in Peripheral blood smear in image are denoted with *blue arrow* and *star mark* shows small lymphocyte. It is predominantly microcytic hypochromic RBC morphology (Leishmanstain,x 400). Inset image showing fragmented RBCs with Sickled RBCs (Field stain, x 1000).

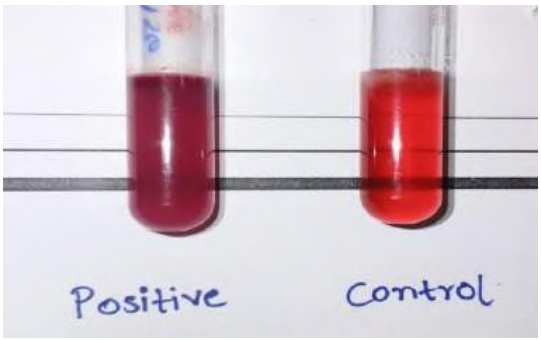

Figure 2: Sickling solubility (tube) test

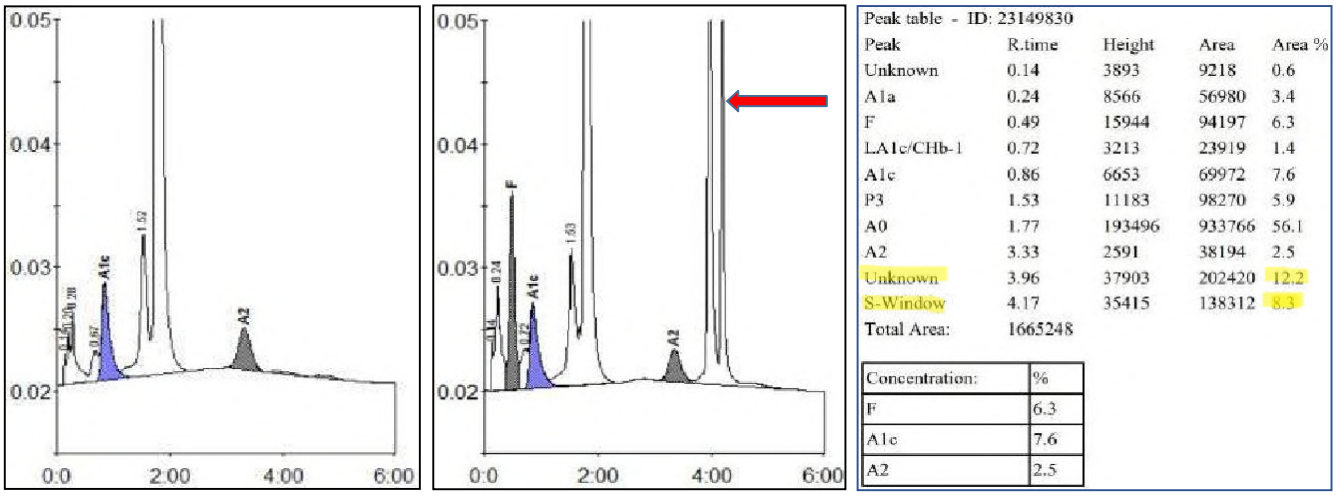

Figure 3: HPLC: First graph showing HPLC of normal person (control) and second graph showing HPLC of the patient with red arrow depicting HbS and Unknown windows. The percentage of Hb variants are depicted on the right.

For screening purpose, HPLC was performed (Figure 3) on Bio Rad D10 and the result revealed two distinct peaks; one for HbD in the Unknown window and another for HbS within S window.[1] This fresh EDTA blood sample was tested for HPLC after applying HPLC Biorad controls. The later showed compatible results.

This HPLC EDTA-sample was run again after doubling the sample volume showing concordant Hemoglobin variants. Next day to confirm the same, fresh EDTA sample was tested without any variations in HPLC results. Fridge stored (4 degree celsius) sample was not used for testing in our case.

From the blood investigation reports, it was found that patient had low hemoglobin and blood transfusion was done. The patient was given five pints of Red cell transfusions during her hospital stay of 12 days with iron chelation by medicine consultants. At the time of discharge, the patient's hemoglobin was 9.3 gm% and was asked for follow up after 15 days. Multi-vitamin supplements were given to her on discharge to prevent further ensuing deficiency anemia.

Parental and her children's HPLC testing were advised. She was advised Genotyping study for Hemoglobinopathy at higher center.

DISCUSSION

Out of the more than a dozen variations of hemoglobin D (HbD), HbD-Punjab is the most prevalent. Rarely, a HbD compound heterozygous condition with S-window has been reported.[1]

Haemoglobin chains-encoding genes are mutated in about 7% of the world's population. Genetic changes in the globin can impact the rate at which hemoglobin is produced overall, or they can produce multiple hemoglobin variations by changing the molecular makeup between hemoglobin. One of the variation that is Hb-D stemming from a point mutation in the human beta-globin gene (HBB) within the first base of the codon 121 (GAA_CAA), with the replacement of glutamic acid for glutamine (Glu-Gln).[4]

Hemoglobin D-Punjab mainly occur in Sikhs in Punjab (2%), followed by Gujarat population and those belonging to these sects that emigrated to other states (1%).[4]

Most often, HbA/D is discovered unintentionally during routine HPLC screening of parents whose children have Hb DP syndromes, prenatal screening, and community screening initiatives as it is inherited as autosomal recessive trait. When individuals are assessed for moderate to severe anemia, homozygous and compound heterozygous

forms are found. The benefit of CE-HPLC is that it can precisely quantify these aberrant hemoglobins in addition to quickly identifying them, just like in our case.[5,7]

In our laboratory, a Hb variant is identified by HPLC by comparing its RT (Retention Time), amount as a percentage of total Hb, and peak characteristics with the Bio-Rad Library of Variants, as well as the case's ethnic origin. For screening purpose, HPLC was performed on Bio Rad D10 and the result revealed two distinct peaks; one peak for HbD in the Unknown window at retention time of 3.96 minutes, which included 12.2% of total Hb, and another peak for HbS within S-window at retention time of 4.17 minutes, which included 8.3% of total Hb. HbF peak was 6.3% in our case (increased for age). HbA2 was 2.5% in our case.[6]

HbS occurs due to mutation in beta globin chain of hemoglobin (glutamic acid is replaced by valine at 6th position). Various double heterozygous state for HbS and a second disorder of Hb synthesis results into various clinical and hematological manifestation which can mimic as sickle cell anemia. Other combination of sickle cell disease are HbSC disease, HbS/HPFH, HbSE disease, Hb SO- Arab disease.[8]

Hb-D is a rare condition. Except for HbSD, all the other presentation of Hb-D are clinically benign. HbSD has a variable clinical presentation. Anemia and sickle cell crises are quite common.[1]

The benefits of CE-HPLC include quick detection and precise quantification. Heterozygous form of Hb-D is clinically asymptomatic.[5] But when a person has HbD and HbS trait together it will be producing clinically significant symptoms and complications. Double heterozygosity for HbD and HbS results in moderately severe clinical presentation of sickle cell disease with increased reticulocyte count.

CONCLUSIONS

Hemoglobinopathies are disorders that are inherited. After routine CBC, PBS and reticulocyte count testing, a clinico-hematological opinion of hemolytic anemia should be formed. HPLC as a screening tool must be done in hemolytic anemia cases with consanguineous marriages with respect to certain susceptible Indian communities. This will help in prompt detection of HbS and HbD hemoglobin defects presenting as compound heterozygous state.

Early diagnosis helps in prompt inception of adequate timely therapy. Red cell transfusions help to resurrect Hemoglobin levels to acceptable clinical goal limits between 9 to 10 gm% in hemolytic anemia cases. Familial genetic testing is important in such cases born out of consanguineous marriages.

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