

# Unveiling complex interaction of sickle cell hemoglobin with another hemoglobinopathy – A Rare Entity



CASO CLÍNICO

Neha Singh<sup>1</sup>, Garima Baweja<sup>2</sup>, Akanksha Bhatia<sup>3</sup>, Vijay Kumar<sup>4</sup>.

<sup>1</sup> Senior resident, Department of Pathology, Atal Bihari Vajpayee institute of medical sciences and Dr. Ram Manohar Lohia Hospital, New Delhi, India

<sup>2</sup> Consultant Pathologist, Department of Pathology, Atal Bihari Vajpayee institute of medical sciences and Dr. Ram Manohar Lohia Hospital, New Delhi, India

<sup>3</sup> Assistant Professor, Department of Pathology, Atal Bihari Vajpayee institute of medical sciences and Dr. Ram Manohar Lohia Hospital, New Delhi, India

<sup>4</sup> Professor, Department of Pathology, Atal Bihari Vajpayee institute of medical sciences and Dr. Ram Manohar Lohia Hospital, New Delhi, India

vijaypgi1@gmail.com

Fecha recepción: 2/11/2023

Fecha aprobación: 2/5/2024

HEMATOLOGÍA

Volumen 28 n° 1: 77-82

Enero - Abril 2024

**Keywords:** Sickle cell, Hb S/D<sup>Punjab</sup>, hemoglobinopathy, high performance liquid chromatography.

## ABSTRACT

Hemoglobinopathies constitute one of the most common inherited hematological disorders in the world with an increasing global disease burden each year. One among them is sickle cell disease with diverse genotypes and wide phenotypic heterogeneity. Many subgroups exist within the umbrella of sickle cell disease. Hb S/D<sup>Punjab</sup>, a rare hemoglobinopathy, is one of them, mimics sickle cell disease, and is discussed in the present study. We describe one such unusual clinical case of a young child who presented with intermittent fever and joint problems. The study case was found to have Hb S/D<sup>Punjab</sup> by high

performance liquid chromatography. Clinical and hematological details of this rare condition is only briefly discussed in the literature. Precise diagnosis can be made using high performance liquid chromatography in conjunction with family studies.

## INTRODUCTION

Hemoglobinopathies constitute a major burden of the inherited hematological disorders worldwide. Sickle cell disease (SCD) constitutes one such disorder caused by a structural variant of hemoglobin that damages and deforms red blood cells and affects multiple organ systems. It is categorized as SS,

SC, SD<sup>Punjab</sup> (Los Angeles), SO-Arab, S- $\beta$ -thalassemia, S-hereditary persistence of fetal hemoglobin and SE<sup>(1)</sup>. Among these, S/D<sup>Punjab</sup> is a rare compound heterozygous hemoglobinopathy characterised by interaction of Hb S with Hb D-Los Angeles (Hb D<sup>Punjab</sup>) and the coexistence of two globin gene variants: HBB 6(GAG>GTG) and HBB 121(GAA>-CAA)<sup>(2)</sup>. Clinically, these patients present with a variable course and often mimic sickle cell anemia. Hb D<sup>Punjab</sup> comigrates with Hb S on alkaline electrophoresis gel, making this mutation look like SCD and causes moderate to severe chronic hemolytic anaemia as both beta chains are involved with presence of splenomegaly in many cases. Some patients have severe vaso-occlusive complications<sup>(3)</sup>. We present here one such rare case diagnosed on high performance liquid chromatography (HPLC) and confirmed by parental studies.

## CASE REPORT

A 12-year-old male patient came to pediatric out patient department complaining of intermittent fever and multiple site-joint pain and swelling involving bilateral knees, ankles, elbows and wrists since 2 days. Patient also reported easy fatigability and joint pain associated with tenderness due to which he was not able to stand. Patient had history of similar episodes in the past since age of 2 years which followed the pattern of 3-4 episodes per year lasting for few days. On clinical follow-up, he presented with innumerable painful crises. On examination, patient was found to be pale and icteric. Ultrasound abdomen showed hepatosplenomegaly with presence of multiple subcentric hyperechoic foci in spleen. Liver and spleen size were 13.8 cm and 11.4 cm, respectively. Magnetic resonance imaging of right ankle revealed marrow reconversion in right foot bones with multiple foci of osteonecrosis in distal tibia, fibula, talus, calcaneum, cuneiforms, cuboid and proximal aspects of 1<sup>st</sup> to 4<sup>th</sup> metatarsals and complete osteonecrosis of navicular bone. His initial hematological workup (Table 1) showed microcytic hypochromic anaemia with moderate to marked red cell anisopoikilocytosis with presence of sickle cells, target cells and fragmented RBCs in peripheral smear (Figure 1A). Reticulocyte count was 5.7%. Liver function tests revealed mildly elevated total and indirect bilirubin as 3.7 mg/dL and 2.81 mg/dL, respectively. Sickling test was carried out which came positive (Figure 1B). Possi-

bility of sickle cell disease was suggested and high performance liquid chromatography (HPLC) was advised. No history of similar complaints in the family members was reported. HPLC graph showed major hemoglobin peak in D window with a second prominent peak of Hb S (Twin peak sign) and no peak at Hb A position. HbF was elevated (18.9%) and HbA2 was reduced (1.9%). It was suggestive of compound heterozygous state for Hb D<sup>Punjab</sup> and hemoglobin S or hemoglobin S/D (Hb S/D) disease (Figure 2A). Family screening and DNA analysis were advised for confirmation since diagnosis of Hb D<sup>Punjab</sup> was presumptive based on HPLC analysis (Figure 3). HPLC of father showed prominent peaks of Hb A and Hb S, the pattern was consistent with heterozygous state for Hb A/S (Figure 2C), whereas of mother showed prominent peaks of Hb A and an abnormal hemoglobin within Hb D retention time, suggestive of heterozygous state for Hb A/D<sup>Punjab</sup> (Figure 2B). HPLC of the patient's younger brother showed that he was also heterozygous for HbA/S (Figure 2D). Hemoglobin electrophoresis along with family history was suggested for confirmation in all these cases. While hemoglobin electrophoresis may suffice for relatives from a single line of ancestry (paternal or maternal) in routine practice, it also becomes crucial to conduct the sickling test for descendants displaying any anomalous bands in electrophoresis. Patient was started on treatment with hydroxyurea and folic acid.

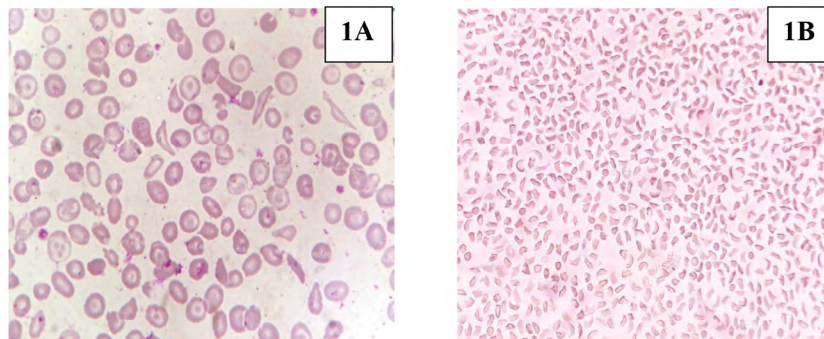
## DISCUSSION

Hemoglobinopathies are the most common genetic diseases, affecting approximately 7% of the world's total population<sup>(4)</sup>. More than 300,000 children are born each year with inherited hemoglobin disorders and approximately 80% among these are from mid to low-income countries<sup>(5)</sup>. Sickle cell disease (SCD) is currently the most prevalent severe monogenic condition in the world, with significant prevalence in sub-Saharan Africa, the Middle East, areas of the Mediterranean, and India. In South Asia region, India has the greatest disease prevalence accounting to around 20 million cases<sup>(6)</sup>. SCD involves a group of hereditary diseases that affect haemoglobin by a single nucleotide change at position 6 of the  $\beta$ -globin gene. Clinical symptoms arise from the polymerization of the resultant sickle-shaped variation of haemoglobin (Hb S), which sets off a series of changes in RBCs. Both acute and chronic consequences cause

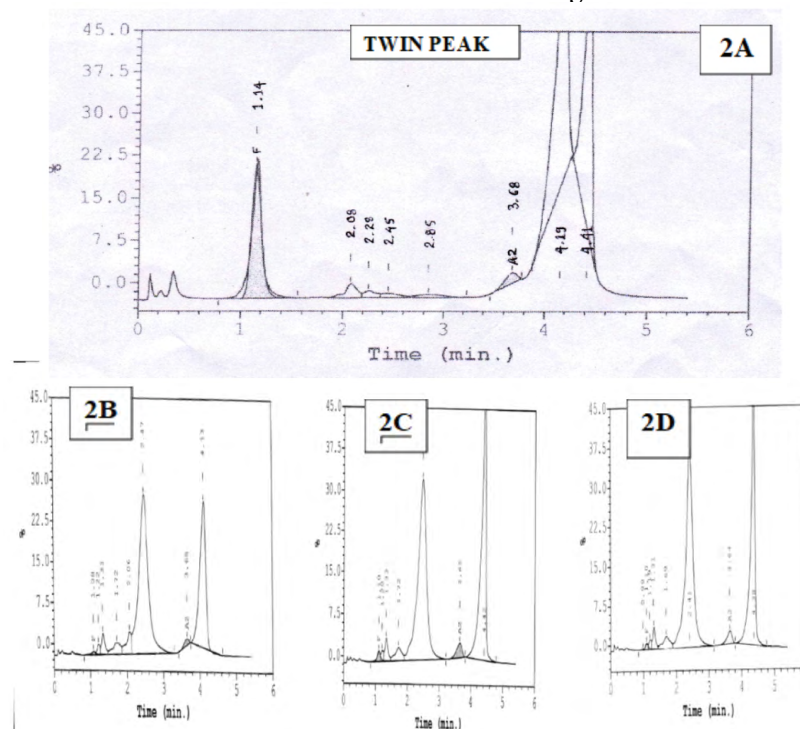
**Table 1.** Complete blood count parameters

CBC parameter	Value
Hemoglobin (g/dL)	8.8
RBC count (million/ $\mu$ L)	3.61
HCT (%)	27.5
MCV (fl)	76.2
MCH (pg)	24.4
MCHC (g/dL)	32.0
RDW%	22.6
TLC (per $\mu$ L)	5000
Platelets (per $\mu$ L)	1,50,000

**FIGURE 1. A.** Peripheral smear of case showing microcytic hypochromic RBCs with many sickle cells and few target cells. **B.** Positive sickling test using 2% sodium metabisulfite



**FIGURE 2. Haemoglobin HPLC chromatogram. A.** Case showing Twin peak sign characteristic of Hb S/D<sup>Punjab</sup> variant **B.** Mother of case showing heterozygous state for Hb D<sup>Punjab</sup> **C.** Father of case showing sickle cell trait. **D.** Brother of case showing sickle cell trait



significant morbidity in these patients<sup>(7)</sup>. The most severe condition in the clinical spectrum of SCD is sickle cell anemia characterized by frequent vaso-occlusive crises with early death in exceptional cases. Intermediate in severity are S/D, S/C, and sickle cell beta thalassemia. The main clinical feature of hemoglobin S/D (Hb S/D) disease is a moderately severe hemolytic anemia, characterized by persistent, intermittent episodes of jaundice and bone pain. Increase in hemolysis and jaundice was noted in association with infection and pregnancy. The distinction between sickle cell anemia and Hb S/D disease is important because of different prognosis in the two diseases<sup>(8)</sup>. Although there is a wide range in the disease phenotype, the majority of patients with Hb S/D disease present with severe disease. Apart from that, Hb F levels in these patients is not associated with disease severity, instead play a protective role<sup>(2)</sup>.

Itano first described Hb S/D in 1951 in a Caucasian family as hemoglobin D-Los Angeles. Hemoglobin D was the fifth hemoglobin to be described. Biochemically, four types of hemoglobin D have been described, D-alpha thalassemia, D-beta thalassemia, D-beta<sup>Punjab</sup> and D-beta Ibadan. Among these, Hb D<sup>Punjab</sup> is the most common. In a report of structural hemoglobin variants, Hb D<sup>Punjab</sup> constituted 0.55% (38/6889) of all screened samples. Of these, double heterozygous Hb S/D<sup>Punjab</sup> cases constituted 0.03% (3/6889)<sup>(9)</sup>. Patients homozygous for haemoglobin D do not exhibit sickling, hence the process of sickling in heterozygous Hb S/D disease patients is somewhat similar to those with sickle cell anaemia. According to proven studies, beta chains of haemoglobin S form a hydrophobic bond when the oxygen tension is low. This bond extends from the abnormally substituted valine at the sixth position of N-terminus of one beta chain to the N-terminal amino acid of another beta chain along with the formation of paracrystalline aggregates, distorting the cell shape like a sickle<sup>(8)</sup>.

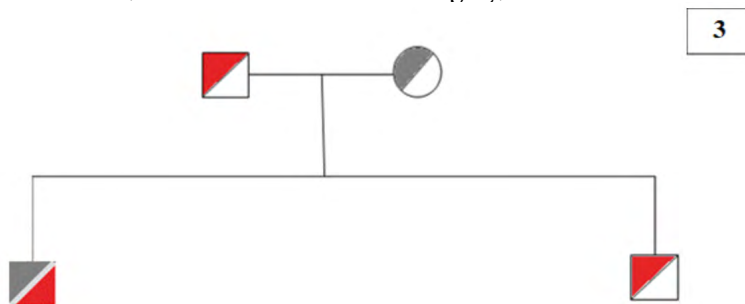
Interaction of Hb S with Hb D<sup>Punjab</sup> is an uncommon hemoglobinopathy with a variable clinical expression. Our patient was a young adolescent boy who presented with severe anemia, fever and multiple-site joint pain with history of similar complaints in the past since the age of 2 years. On examination, there was marked pallor and presence of hepatosplenomegaly. No previous history of jaundice or blood transfusion or easy bruising from any sites in the past was documented. However, in the current

visit, 1 unit packed RBC was transfused. With the above clinical picture, following clinical possibilities were suggested – hemolytic anemia, systemic onset juvenile idiopathic arthritis, malignancy and acute renal failure. On investigations, serum iron, ferritin, vitamin B12, folic acid, vitamin D3, rheumatoid factor, ESR, ECG findings, C3, C4 levels were within normal limits. Brewer test for G6PD deficiency and direct antiglobulin test were negative. Serum total, direct and indirect bilirubin levels were elevated and parathyroid hormone levels were low. Peripheral smear did not reveal any atypical cells. However, there was presence of many sickle cells along with target cells and fragmented RBCs. Sickling test was advised which came out to be positive following which HPLC was suggested.

HPLC showed elevated Hb F (18.9%) and variant Hb peaks at the D (42.1%) and S (32.2%) windows. Hb A peak was virtually not visible. Based on this result, the diagnosis of sickle cell/haemoglobin D<sup>Punjab</sup> compound heterozygosity was made. Patient's family was counselled and advised to undergo HPLC. All the family members including patient's father, mother and younger brother were asymptomatic. Patient's father HPLC revealed a peak in the S-window (40.4%) along with Hb A peak (50%) and patient's brother HPLC showed similar result with an S-window peak (37.9%) and Hb A peak (52.3%) indicating sickle cell trait. Patient's mother HPLC showed an abnormal peak in the D-window (31.7%) along with Hb A peak (55.6%), suggesting heterozygosity for Hb A/D<sup>Punjab</sup> (Figure 3).

In Hb S/D disease, the glutamine residue in Hb D<sup>Punjab</sup> facilitates the polymerization of Hb S resulting in a moderately severe hemolytic anemia, characterized by jaundice and bone pain. In 2019, Ali et al reported a single case of Hb S/D from Uttar Pradesh out of 2200 patients screened for HPLC (0.04%)<sup>(9)</sup>. Rohilla et al<sup>(10)</sup>, reported first case of Hb S/D<sup>Punjab</sup> in a 24 years old primigravida, from Punjab during routine antenatal investigation at 13 weeks period of gestation and was under regular follow up. Nogueira et al<sup>(3)</sup>, reported first case of Hb S/D on HPLC in the state of Brazil. Subsequent molecular study of chromosomal polymorphism with betaglobin S gene, revealed the Bantu haplotype. Many studies assessed cohorts of Hb S/D<sup>Punjab</sup> patients and observed moderate or severe anemia, as well as complications such as painful crises, gall-

**FIGURE 3.** Family pedigree chart showing inheritance of Hb SD<sup>Punjab</sup>.  
Hb S – Labelled with red, Hb D<sup>Punjab</sup> – Labelled with grey, Hb SD<sup>Punjab</sup> – Labelled with red/grey.



stones, and aseptic necrosis of femoral head, and the need for blood transfusion by some patients<sup>(3)</sup>. Transfusion requirement has been reported to vary from 0 to 80% in different studies<sup>(9)</sup>.

A proper differentiation of these variants is essential to avoid erroneous counselling of these rare clinically important Hb S compound heterozygote patients<sup>(2)</sup>. As a protocol, all samples showing a single band at the hemoglobin S position should be confirmed by sickling test and acid pH electrophoresis in conjunction with family studies. This step is crucial to exclude the possibility of a compound heterozygote. HPLC should be preferred as it is an excellent tool for detection of compound heterozygotes. However, a major limitation of HPLC is that there can be overlap and more than one variants may co-elute within a given retention time. Few other variants such as Hb C-Georgetown and Hb S Memphis also exhibit sickling<sup>(4)</sup>.

Hb D<sup>Punjab</sup> can be readily distinguished from Hb S by its normal solubility, difference in electrophoretic mobility on agar gel at acidic pH and its failure to produce sickling. Hemoglobin S and D may be easily separated by agar electrophoresis at pH 6.2. Some factors affecting variability in clinical manifestation of Hb S/D<sup>Punjab</sup> include co-inheritance of  $\alpha$ -thalassaemia, enhanced Hb F levels and the type of haplotype framework on which  $\beta$ /S is inherited<sup>(9)</sup>.

Since the pathophysiology of Hb S/D<sup>Punjab</sup> is similar to Hb S disease, management guidelines for sickle cell anaemia may be adopted in severe cases of Hb S/D<sup>Punjab</sup> to reduce morbidity and mortality<sup>(10)</sup>.

## CONCLUSION

A proper diagnosis of hemoglobinopathy is essential to rule out its interaction with another sickle cell variants, which may exhibit variable

clinical expression. Accurate delineation of this variant is very important to facilitate an effective response as it may have life threatening complications. All samples showing an abnormal hemoglobin HPLC should be worked up for detailed family history. This step is crucial to detect the presence of an interaction with another hemoglobinopathy. Family studies play an important role for carrier detection, which further help in premarital counselling. In consanguineous marriages, risk of inherited disorders is higher due to shared genetic ancestry, so genetic counseling should emphasize the potential presence of both hemoglobinopathies (such as sickle cell disease) and thalassemias. Testing should include a comprehensive panel for common hemoglobin variants and thalassemia mutations relevant to the specific ethnic background of the family. For non-consanguineous relatives, genetic counseling should focus on the presence or absence of any hemoglobinopathy, whether structural variants like hemoglobin D<sup>Punjab</sup> or thalassemic mutations. Diagnosis of hemoglobin D<sup>Punjab</sup> was presumptive in this study based on HPLC findings and regional prevalence data. Necessity of DNA confirmation should be emphasized in similar cases to ensure diagnostic precision. Hence, it is desirable to do a DNA analysis for definite categorisation of hemoglobinopathy. While DNA testing was proposed to the family, it could not be performed due to financial constraints. Extensive testing, family screening with proper and detailed history and timely diagnosis helps to prevent poor outcome of patient. Preconceptional counselling and possible obstetric complications also have a major role in achieving the best maternal and neonatal outcome. Precise genotype diagnosis aids further in error free counselling and proper management.

**Authors' contributions:** Dr. Neha Singh collected data and formulated the manuscript, Dr. Garima Baweja and Dr. Akanksha Bhatia helped in case report design and its review. Dr. Vijay Kumar provided additional valuable inputs into the study and reviewed the case report.

**Conflictos de interés:** Los autores declaran no poseer conflictos de interés.

### References

1. Rodak., Carr. Clinical hematology atlas. St. Louis: Elsevier; 2017.
2. Hassan M, Ali Al-Barazanchi Z, Abdulateef S, Jaber R. Double heterozygosity for hemoglobin S and D Punjab in Basra, Iraq: A Clinical and hematological study of 42 patients. *Journal of Applied Hematology*. 2019;10(4):134.
3. Nogueira R, Leite C, Souza L, Barbosa A. Clinical and laboratory repercussions in patient with hemoglobin SD-Punjab disease: a case report. *Jornal Brasileiro de Patologia e Medicina Laboratorial*. 2017;53(5).
4. Randolph TR. Hemoglobinopathies (Structural Defects in Hemoglobin). In Rodak BF, Fritsma GA, and Doig K (eds.): *Hematology: Clinical Principles and Applications*, 3rd ed, St. Louis: Saunders, Elsevier, 2007:333-54.
5. Weatherall, D. J. The challenges of haemoglobinopathies in resource-poor countries. *Br J Haematol*. 2011; 154:736-744.
6. Brousse V, Rees D. Sickle cell disease: More than a century of progress. Where do we stand now?. *Indian Journal of Medical Research*. 2021;154(1):4.
7. Hockham C, Bhatt S, Colah R, Mukherjee M, Penman B, Gupta S et al. The spatial epidemiology of sickle-cell anaemia in India. *Scientific Reports*. 2018;8(1).
8. Cawein M. Hemoglobin S-D Disease. *Annals of Internal Medicine*. 1966;64(1):62.
9. Ali W, Jain M, Agarwal S, Kumar A. A Case of Hemoglobin Sickle-D Punjab. *Indian Journal of Hematology and Blood Transfusion*. 2019;36(1):205-207.
10. Rohilla Rakhi, Rohilla M, Malhotra P, Das R, Jain V et al. Compound Heterozygous Hemoglobin SD Disease Presenting as Sickle Cell Crisis in Pregnancy. *Journal Of Woman's Reproductive Health*. 2016; 1(2):25-28.



**Atribución – No Comercial – Compartir Igual (by-nc-sa):** No se permite un uso comercial de la obra original ni de las posibles obras derivadas, la distribución de las cuales se debe hacer con una licencia igual a la que regula la obra original. Esta licencia no es una licencia libre.