

Haemoglobin Sickle D Punjab: - A Case Report

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SUMMARY

Haemoglobin S D-Punjab is a rare compound heterozygous haemoglobinopathy characterised by the presence of two β globin gene variants: $\beta 6(\text{GAG} \rightarrow \text{GTG})$ and $\beta 121(\text{GAA} \rightarrow \text{CAA})$. These patients' clinical and haematological features mimic haemoglobin S disease. We describe the first case of doubly heterozygous HbSD-Punjab from Malaysia managed with regular blood transfusion at the age of one. This case highlights the propensity for occurrence of rare phenotypes within our multi-ethnic population and emphasises the importance of accurate genotyping to avoid erroneous counselling, and to plan an effective patient management strategy before complication evolves.

KEY WORDS:

Haemoglobinopathies; Hb SD-Punjab; Malaysia

INTRODUCTION

Haemoglobinopathies are characterised by structurally abnormal haemoglobin variants of the normal adult haemoglobin (HbA). Haemoglobin S, C, D and E are classical examples of β chain variants. Hb S ($\beta 6(\text{A3})$, Glu \rightarrow Val) variant has a worldwide distribution with highest gene frequencies seen in equatorial Africa, Quatif osases of eastern Saudi Arabia and parts of India. In Malaysia, Hb S variant is seen amongst the Malaysian Indians at a low frequency¹. Hb S heterozygotes (β^S/β^A) are usually asymptomatic but rarely may be associated with clinical and haematologic manifestation of significance. Homozygotes (β^S/β^S), however, has enormous clinical importance. Haemoglobin D Punjab (also known as D-Los Angeles) is characterized by substitution of glutamic acid by glutamine at position 121 of the β -globin chain and is most commonly encountered in northwest India, Pakistan, and Iran; and is asymptomatic both in heterozygous and homozygous states. Co-inheritance of both haemoglobin S and haemoglobin D-Punjab, termed HbSD disease, may manifest with mild to moderate haemolytic anaemia resembling those of sickle cell anaemia.

CASE REPORT

An Indian boy presented with a history of upper respiratory tract infection at the age of 4 months and was noted by his GP to be very pale (Hb of 5.5 gm/dL). He was then referred to a district hospital for further investigation and management. Red cell indices at the hospital revealed the following: Hb 6.5 gm/dL, RBC 2.3 X10⁶/dL, MCV 86.0fL, MCV 27.5pg and

MCHC 32%. Blood smear showed marked anisopoikilocytosis with microcytic hypochromic red cells, dacrocytes, target cells, fragmented and polychromatic cells. A few sickle and boat shaped cells were also seen. Sickling test done was positive. The Hb electrophoresis done at pH 8.5 showed a variant band at haemoglobin S position along with a thick band at HbF position. A presumptive diagnosis of sickle cell disease (SCD) was then made. Further analysis on HPLC showed presence of Hb F and variant Hb peaks at the D (20.8%) and S (19.2%) windows. Hb A peak was virtually not visible. Based on this result the diagnosis of sickle cell/haemoglobin D-Punjab compound heterozygosity was made.

At the age of one, the patient became transfusion dependent requiring two monthly blood transfusions. To establish a definitive molecular diagnosis, patient's blood specimen was sent to our laboratory at Institute for Medical Research. His genomic DNA was tested for β -gene mutations prevalent in our multiethnic population. Having excluded the common point mutations by allele specific primer extension, β -globin gene sequencing was done to detect the presence of other mutations. Sequencing of the β -globin gene read from -100 bp to the 3'UTR showed the co-inheritance of a heterozygous change at codon 6 (GAG \rightarrow GTG) and also a heterozygous mutation at codon 121 (GAA \rightarrow CAA). Thus, the diagnosis of $\beta^S/\beta^{\text{D-Punjab}}$ compound heterozygosity was confirmed. The parent's blood samples were not sent to our institute for testing, therefore we were unable to fully determine the pattern of inheritance. The mother's Hb analysis report was consistent with Hb S trait. However the father's Hb analysis was to have been reanalysed.

DISCUSSION

Several variants of HbD such as Hb D Punjab, Hb D Iran, Hb D Ibadan and Hb D Bushman have been noted to co-inherit with HbS. With the exception of Hb D Punjab, compound heterozygous states of HbS with HbD variants were clinically innocuous. The substituted glutamine residue in Hb D Punjab interacts with Hb S to facilitate polymerisation during deoxygenation, and thus is presented perniciously with clinical and haematological features of variable degree of haemolytic anaemia resembling sickle cell anaemia². Studies from India, Pakistan, Iran, UAE and Mexico have shown similar clinical presentations for HbSD-Punjab mimicking severe form of sickle cell anaemia³.

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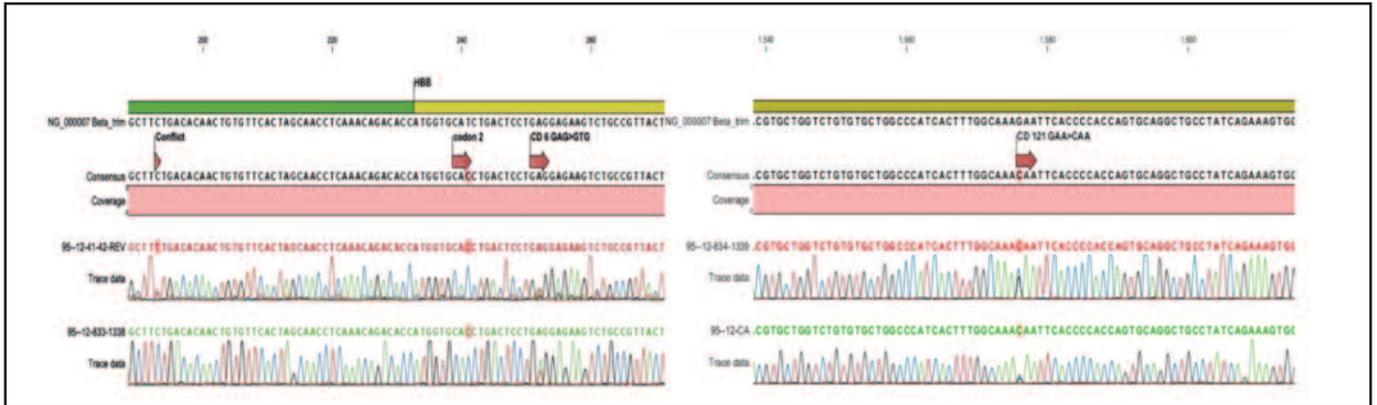


Fig. 1 : The sequence trace of the β globin gene above shows the codon 6 (GAG→GTG) and codon 121 (GAA →CAA) mutation inherited by the patient.

Accurate delineation of these variants is very important to facilitate an effective response to life threatening complication and to avoid erroneous counselling of these rare clinically important Hb S compound heterozygote patients. As a rule of thumb, all samples showing a single band at the haemoglobin S position on conservative electrophoresis at alkaline pH should be retested by an alternative technique either by citrate agar/acid gel method, or by IEF. This step is crucial to exclude the possibility of a compound heterozygote. Alternatively, HPLC should be preferred if available. Care must be taken if considering a diagnosis on HPLC chromatograph as it is possible for more than one variant to overlap and co-elute within a given retention time window. Haemoglobin eluting in the HbS window should have a sickle solubility test performed to confirm the presence of sickling haemoglobin. It should be noted, however, that positive sickling test alone indicates the presence of a sickling haemoglobin and does not provide definitive diagnostic information on the identity of the haemoglobin or which other haemoglobins may be present. Erythrocytes containing other variant haemoglobins in which $\beta^{(Glu \rightarrow Val)}$ is present, such as HbC-Georgetown and Hb S-Memphis also exhibit sickling. The sickle cell solubility test may not be reliable at HbS level below 15-20%, reflecting that the test is unreliable in neonates, infants and in recent transfusion with HbAA blood.

Hb D-Punjab can be readily distinguished from Hb S by its normal solubility, difference on electrophoretic mobility on agar gel at acidic pH and its failure to produce sickling.

Some factors affecting variability in clinical manifestation of Hb SD-Punjab may include co-inheritance of α -thalassaemia, enhanced HbF levels and the type of haplotype framework on which β^s is inherited. The coexistence of α thalassaemia and sickle cell anaemia does have a proven affect on the phenotype reflected by less haemolysis. Moreover, it is known that Bantu and Benin haplotypes of β^s are associated with the clinically severe sickle cell disease. However, unlike in HbSS syndrome, elevated levels of HbF concentration do not seem to ameliorate the clinical phenotype of HbS D Punjab⁴.

As the pathophysiology HbS D-Punjab is similar to HbS disease, adopting the guidelines proposed for sickle cell anaemia to manage this clinically significant phenotype will be critical to reduce morbidity and mortality⁵.

CONCLUSION

According to our extensive literature review and to the best of our knowledge, this is the first case of HbSD-Punjab reported from Malaysia. Haemoglobin variant such as HbS and HbD are extremely rare in the multi-ethnic Malaysian population. However, demographic changes such as population migration, miscegenation causes new spectrum of inherited haemoglobin disorders to emerge. Therefore, it is important to make a precise genotype diagnosis to facilitate error free counselling and proper management HbS D disease. A combined data from HPLC, electrophoresis at alkaline and acid pH, and the sickle solubility test enable definitive identification of HbA, HbF, HbS, HbC, and several others rare variants.

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