

Correlation of HbF, HbA₂, and HbS in sickle cell disease and its prevalence in Nigerian patients: A case series of 2 patients

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ABSTRACT

Sickle cell disease (SCD) is the most common inherited disorder of hemoglobin worldwide. In Nigeria, the prevalence of SCD is 20–30/1000 live births. The burden of the disease has reached a level where it contributes 9–16% of the under-five mortality in many West African countries. This case series evaluated the chromatographic patterns and red blood cell indices of sickle cell homozygous patients. Red cell indices, blood film, sickle solubility test, and chromatographic patterns using Bio-Rad HPLC D10 were evaluated for both patients. Both the patients were Nigerian and HPLC showed HbS window 81.7 and 81.6% and increased HbF, that is, 7.5 and 8.8%. HbA₂ was normal in both the cases, that is, 2.2 and 2.6%. Our data suggest that homozygous sickle cell disease is very common among the Nigerian population with an increase in HbF along with HbS and HbA₂ is normal.

Key words: Blood disorder, Nigerian, Sickle cell disease

Sickle cell disease (SCD) is an inherited blood disorder characterized by clinical heterogeneity that may be influenced by several factors including environmental, ethnicity, race, social, economic, genetic, and epigenetic factors [1]. SCD causes significant morbidity and mortality and affects the economic and healthcare status of many countries [2]. In SCD the problem typically begins around 5–6 months of age. SCD accounts for about 70% of the world's major hemoglobinopathies [3]. The frequency of sickle cell anemia in Nigeria is about 20 per 1000 births, this is due to the high prevalence of sickle cell trait, that is, 23.7% [4]. Various methods are used by laboratories in the evaluation of hemoglobin disorders [5].

Here, we present the case series of two patients who presented with a complaint of pain abdomen in a hospital of Greater Noida. This case series evaluated the high-performance chromatographic patterns and red blood cell indices of sickle cell homozygous patients in the Nigerian population.

CASE SERIES

Case 1

A 20-year-old male presented with a chief complaint of pain abdomen and fatigue. There was no medical history. On general


examination, the patient was not stable. Local examination showed an enlarged spleen. His ultrasound showed moderate splenomegaly.

Case 2

A 19-year-old male presented with a chief complaint of fatigue. General examination was unremarkable. Local examination showed moderate splenomegaly which was also confirmed by ultrasound examination.

The blood investigations of both patients are shown in Table 1. Four milliliters of EDTA blood were collected from both the patients and a reticulocyte smear was made. Following this, complete blood count (CBC), peripheral smear (PS) along with sickling test, and high performance liquid chromatographic (HPLC) technique were performed in both the patients. CBC and reticulocyte count were analyzed using six parts XN-1000 hematology analyzer. A sickling test was performed using 2% sodium metabisulfite. Results of sickling were read after 2 h and 24 h, respectively. D10 machine was used for HPLC analysis.

Peripheral smear in both the patients showed sickle cells (Fig. 1). Sickle cells in both the patients were identified on reticulocyte count. Reticulocyte count was increased for both, that is, 10.5% and 8.5%, respectively (Fig. 2). The sickling test was positive for both the patients confirming the presence of hemoglobin S (Fig. 3). CBC revealed low hemoglobin for both

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the patients, which was <11. RBC indices were normal for both the patients. HPLC was performed for both the patients who showed predominantly HbS window accounting for 81.7% and 81.6%, respectively (Fig. 4), and increased HbF, that is, 7.5 and 8.8%. HbA₂ was normal in both cases, that is, 2.2 and 2.6%.

Since both the patients were Nigerian, they were lost to follow-up.

DISCUSSION

Sickle cell disease is one of the common genetic diseases worldwide and it is of high prevalence in Middle-east, Mediterranean region, Southeast Asia, and Sub-Sahara Africa. Sickle cell diseases consist of a group of disorders characterized by the presence of sickle hemoglobin. It is a genetic abnormality involving hemoglobin. Although, it is primarily a red cell disorder, the white blood cells

Table 1: CBC indices and HPLC of both the patients

Indices	Patient 1	Patient 2
Hemoglobin (g/dl)	10.80	10.2
Hematocrit (g/dl)	29.80	28.8
Red blood cells	3.67	3.55
Mean corpuscular volume (MCV) (fl)	81.2	81.1
Mean corpuscular hemoglobin (MCH) (pg)	29.40	28.7
Mean corpuscular hemoglobin concentration (MCHC) (g/dl)	36.20	35.4
White blood cell	13.40	11.09
HbF	8.8	7.5
HbS	81.6	81.7

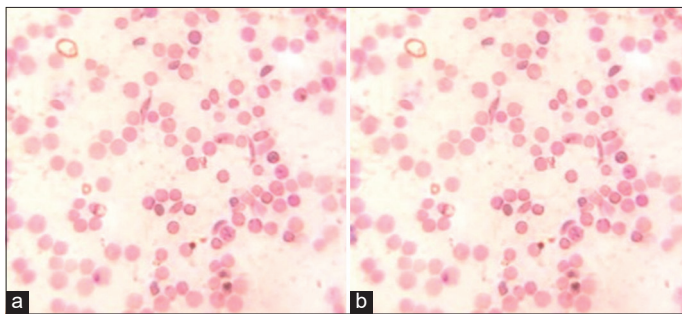


Figure 1: Peripheral blood film showing sickle cells at 100x (arrow heads) (a) Case 1; (b) Case 2

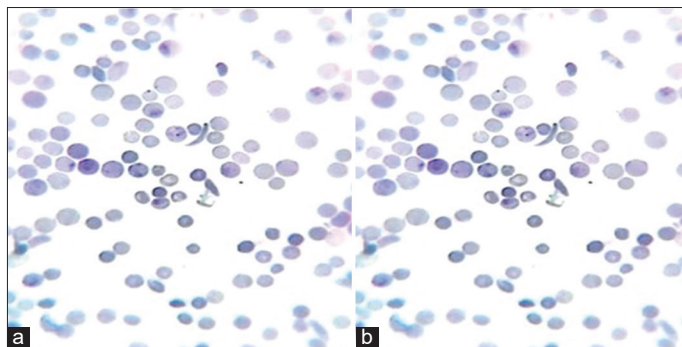


Figure 2: New methylene blue stained smears showing increased reticulocyte count with presence of sickle cell at 100x. (a) Case 1; (b) case 2

and platelets are also affected by the mutation. The consequent hemoglobin S causes polymerization of hemoglobin resulting in hemolysis and anemia.

Hematological characteristics and clinical severity of SCD are heterogeneous and are associated with environmental and genetic factors that include variation in HbF level, the haplotype locale that is linked to the β-globin gene, and the co-inheritance of α-thalassemia, and other Hb variants [6]. Higher expression of HbF in adulthood ameliorates morbidity and mortality in SCD, as an increased level of HbF has been observed to have a beneficial effect in sickle cell anemia due to the inhibition of polymerization of HbS which results in erythrocyte sickling. Full blood count and the red cell indices are essential in the preliminary investigation of hemoglobinopathies. Sickle solubility test detects the presence of hemoglobin S by precipitation of the insoluble hemoglobin variant creating a cloudy, turbid suspension in a prepared test solution.

The mean white blood cell (WBC) count of both patients was 12.24 which denote leukocytosis. This finding was in close association with a case–control study done by Akinbami *et al.* [7]. It was proposed that pain may be responsible for the leukocytosis seen in sickle cell anemia [7]. The mean MCV in both the patients was 81.7% which was is in agreement with Akinbami *et al.* who reported 81.52 fL [7].

A high level of HbS of about 80–90% seen in homozygous disease is associated with a worse disease while the presence of

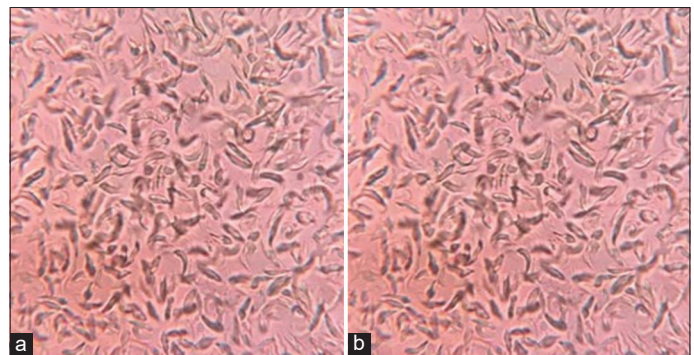


Figure 3: Positive Sickling test with 2% sodium metabisulfite. (a) Case 1; (b) Case 2

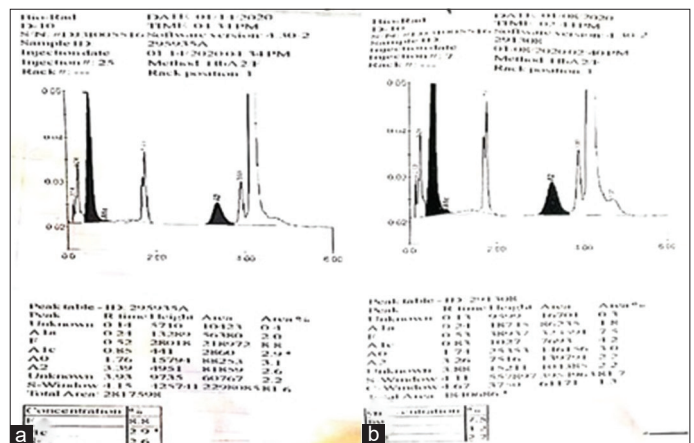


Figure 4: HPLC showing predominantly HbS window (a) 81.7% and HbF 7.5% (case 1); (b) 81.6% and HbF 8.8% (case 2)

alpha thalassemia (one or two gene deletions) ameliorates the disease. Sherley *et al.* in their study stated that the individuals with two copies of HbS develop sickle cell disease with their capillary electrophoretic patterns results typically show 90 to 95% HbS, no HbA, and often slightly elevated HbF in the 5 to 10% range [8]. Our study is in close agreement with this finding.

CONCLUSION

This case series shows raised HbF along with HbS in patients with sickle cell disease and this is very common among the Nigerian population. Thus, a presumptive diagnosis of hemoglobinopathy in Nigeria should transcend routine alkaline electrophoresis and solubility test and should include evaluation of full blood count, red cell indices, iron studies, and HPLC as a method suited for the identification and quantification of hemoglobinopathies.

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