ORIGINAL RESEARCH ARTICLE

HAEMOGLOBIN SO-ARAB IN A CHILD FROM SOUTHWESTERN NIGERIA: CASE REPORT AND REVIEW OF LITERATURE

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SUMMARY

Haemoglobin (Hb) SO-Arab, a compound heterozygous haemoglobinopathy comprising of Hb S and O-Arab, has not been reported previously in Nigeria. This report describes an 18-month old girl from South-western Nigeria diagnosed by high performance liquid chromatography (HPLC) and reviews information about the geographic distribution, clinical and haematological characteristics of patients with Hb SO-Arab.

At the age of five months, the child who was initially diagnosed as having Hb SC genotype by alkaline cellulose acetate electrophoresis, experienced recurrent severe haemolytic crisis, severe sepsis, early onset dactylitis and some other clinical and haematological phenotypes similar to homozygous Hb S disease.

Presence of severe disease in a child diagnosed as Hb SC by cellulose acetate alkaline electrophoresis should alert clinicians to the possibility of another haemoglobinopathy such as Hb SO-Arab. Hence, to make accurate diagnosis of haemoglobinopathy in the absence of gene-based analysis, it may be necessary to combine HPLC with the more frequently used alkaline cellulose acetate and/or acidic citrate agar electrophoresis.

Keywords: Child, Haemoglobin SO-Arab, Nigeria

INTRODUCTION

Most structural haemoglobin (Hb) variants result from single amino acid substitution due to point mutations, in genes encoding the globin chains.[1,2] On rare occasions, they result from multiple amino acid substitutions, deletions, anti-termination mutations and altered posttranslational processing.[1] As at September 2014, Globin Gene Server database (http://globin.cse.psu.edu/) had a total of 1,198 haemoglobin variants.[2,3] Majority of these variants are clinically asymptomatic, however some are associated with variable degrees of pathology. HbS is the most common variant globally and could occur in homozygous fashion (Hb SS) as sickle cell anaemia, or in combination with another variant such as HbC, D-Los Angeles (also known as Hb D-Punjab), Hb E, Hb O-Arab and others.[4]

Haemoglobin SO-Arab is a rare compound heterozygous haemoglobinopathy.[5] While HbS results from replacement of hydrophilic glutamic acid by hydrophobic valine at codon 6 (β6 Glu →Val, GAG>GTG), in Hb O-Arab, lysine replaces glutamic acid at codon 121 (β121 Glu→Lys,

GAA>AAA). At alkaline pH, the electrophoretic mobility of Hb O-Arab on cellulose acetate is similar to Hb C, E, A_2 and also close to Hb S at acidic pH on citrate agar.[5] However, it separates easily on anion or cation exchange chromatography.[5,6] Hence accurate diagnosis of Hb SO-Arab will require both cellulose acetate and citrate agar electrophoreses or high performance liquid chromatography (HPLC). Confirmation using gene probes may also be required.

Hb O-Arab homozygotes are usually asymptomatic, although they may have mild compensated haemolytic anemia with transient jaundice and splenomegaly especially during acute stress, pregnancy, severe infection and chronic kidney disease.[7-9] Heterozygotes (Hb AO-Arab) typically do not have any clinical or haematological abnormality. Individuals with Hb SO-Arab exhibit a clinical course similar to that of Hb SS because Hb O-Arab enhances the sickling effect of Hb S.[5,10]

Despite the huge burden of SCD in Nigeria, there has been no reported case of Hb SO-Arab. This may be due to its geographic distribution or possibly due to the low specificity of alkaline cellulose acetate electrophoresis, which is routinely used to diagnose SCD in Nigeria. This report, thus describes the clinical and laboratory characteristics of a Nigerian child with this rare haemoglobin genotype. It also emphasises the importance of HPLC and family studies in haemoglobin confirmation and management of rare haemoglobinopathies.

CASE REPORT

O.D, a female, initially presented at the Paediatric Haematology Unit of the Wesley Guild Hospital, Obafemi Awolowo University Teaching Hospitals Complex, Ilesa, Osun State, Nigeria in July, 2018 at the age of five months with fever, pallor and yellowness of the eyes. She was the only child of married parents. She had soft tender hepatomegaly of 8 cm and other features of congestive cardiac failure, though she had no splenic enlargement. Her haematocrit level was 17% and alkaline Hb electrophoresis was reported as Hb SC. She was subsequently transfused, discharged and followed up in the clinic.

Four months later, she presented at a private haematology laboratory for repeat haemoglobin electrophoresis which also came out as Hb SC. Her steady-state complete blood count showed anaemia (haematocrit – 22%); leukocytosis (total while blood cell count - 21,200 cell/mm^3) and thrombocytosis (platelet count - 663,000cells/mm^3). She had microcytosis and hypochromia with a mean corpuscular volume (MCV) of 61.8fL and a mean corpuscular haemoglobin (MCH) of 21.5pg. In addition, she had hyperbilirubinaemia with a total serum bilirubin of 135µmol/L (8.2 mg/dL).

At the age of 10 months, she presented again in our facility with severe anaemia and haematocrit was 14% at this time. She received packed red cell transfusion. HPLC Hb quantitation done three months after showed Hb S of 57.6%, A_2 of 1.7%, O-Arab of 32.4% and Hb F of 8.3%. Hb C fraction was 0%. Other clinical phenotypes observed in this child included the following: severe bacterial infection (*Klebsiella spp* and *Staphylococcus aureus* sepsis) at age of 5 and 16 months respectively; and dactylitis/ hand-foot syndrome at age 16 months. She has since been commenced on hydroxyurea therapy and is on routine follow up clinic visit.

HPLC was subsequently done on both parents. The father showed: Hb A = 32.8%, Hb S = 0%, Hb A_2 = 1.4%, Hb F = 0.5%, Hb A1C = 4.7%, Hb O-Arab = 35.7% and altered Hb = 24.9% while the mother had Hb A = 51.6%, Hb A_2 = 5.1%, Hb S = 37.1%, Hb F = 0.8% and Hb A_1C = 5.4%. The complete blood counts and red cell indices of the parents were within normal limits.

The father of the child was a 38-year-old civil servant, Igbira by tribe, from Okene, Kogi State, North Central Nigeria. His parents were said to have migrated from North West, Nigeria. The mother was a 33-

year-old civil servant, a Yoruba from South West Nigeria. The parents of the patient were not into consanguineous marriage.

DISCUSSION

This report, to our best knowledge, is the first on Hb SO-Arab from Nigeria. Globally, only few cases have been reported. Hafsia *et al* reported 20 cases of O-Arab from Northern Tunisia; 16 of whom had co-inheritance with beta thalassemia while the remaining four were in homozygous form, none was co-inherited with S gene.[11] In Ivory Coast, Sangaré *et al* reported 44 individuals with O-Arab which were in the form of AO-Arab, CO-Arab and SO-Arab with the latter running a severe course like in Hb SS.[12]

Patients carrying the Glu-β6 → Val (β^S) on one chromosome and Glu-β121→ Lys ($\beta^{O\text{-}Arab}$) or Glu- β121 → Gln ($\beta^{D\ Los\ Angeles}$) on the other, experience a severe disease.[13] It has therefore been hypothesized that the Glu-β 121 of Hb S plays an important role in gelation and probably weakens the axial $\alpha_2\beta_1$ contacts between Hb S polymers, and the opposite happens when it is substituted by another amino acid.[13] This would explain the clinical severity of Hb SO-Arab and Hb SD-Los Angeles compound heterozygotes.[2] The 13 patients with Hb SO-Arab that presented at the Duke University Medical Center in the United States of America had features of severe SCD such as recurrent severe haemolytic and painful crises, dactylitis, acute chest syndrome, gallstones, aplastic crises, avascular necrosis of head of femur, chronic leg ulcers, osteomyelitis and evidence of end organ damage such as nephropathy, retinopathy and cerebrovascular accident.[5]

Although a rare disease, misdiagnosis may also contribute to the rarity in many developing countries such as Nigeria. Not surprisingly, our patient was misdiagnosed as having Hb SC until HPLC was done. The diagnosis of Hb SO-Arab requires electrophoresis on both cellulose acetate and citrate agar since Hb O-Arab co-migrates with Hb C at alkaline pH, while in acidic medium it migrates between Hb A and close to Hb S, and could be misdiagnosed as Hb SS. Therefore, though both methods are cheap and fast, they may not confirm the presence of this rare haemoglobinopathy.[14]

Many Hb fractions co-elute on HPLC or co-migrate on isoelectric focusing (IEF), it is therefore crucial that multiple methods be used to confirm suspected haemoglobinopathies.[14] British Society for Haematology recommends that abnormal laboratory screening results of haemoglobin should be confirmed by a different technique that is appropriate for the likely abnormality.[15] Also, College of American Pathologists stated that when HPLC is used as a screening test, all non-A, non-S abnormal haemoglobin variants must be confirmed by an alternative method, including alkaline and acid electrophoresis.[16]

As noted previously, our patient exhibited a clinical course similar to that of SCA. She manifested at the age of five months with severe anaemia and *Klebsiella spp sepsis.* In addition, at various times within a space of about one year, she had severe haemolytic crisis, dactylitis and *Staphylococcal aureus* sepsis. Her haematologic parameters showed microcytosis, hypochromia, relatively low level of HbF, leukocytosis and thrombocytosis. How much iron deficiency contributed to this picture was not determined (iron studies were not done), but is a possibility. Also, low red blood cell indices could be as result of beta thalassemia. Presence of severe disease in infancy in a child diagnosed as HbSC by cellulose acetate alkaline electrophoresis should alert clinicians to the possibility of another haemoglobinopathy such as Hb SO-Arab.

CONCLUSION

The co-inheritance of Hbs S and O-Arab gives a rare severe sickling haemoglobinopathy with laboratory and clinical manifestations similar to those of homozygous SCD. For accurate diagnosis of SCD, including compound heterozygotes, the routinely performed tests (cellulose acetate and or acid citrate

agar electrophoresis) may need to be combined with HPLC. Also, gene-based analysis will need to be introduced for confirmatory diagnosis.

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Study conception and design: SAA; initial drafting of the article: SAA, BGO; critical revision for important intellectual content: all authors; final approval of the version to be published: all authors.

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