CASE REPORT

Increased Awareness and Adoption of High Performance Liquid Chromatography will Impact Diagnosis of Beta-Thalassemia Gene Co-inheritance in Nigerian Population: A Case Report

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ABSTRACT

Background:
Using haemoglobin electrophoresis for the diagnosis of sickle cell disease is fraught with limitations especially when co-inheritance with beta-thalassemia gene is suspected.

Aim and Objectives:
The aim of this case study is to report a 28-year old lady diagnosed as Hb SS, but whose parents had Hb AA and Hb AS phenotypes respectively.

Results:
We report a case of a 28 year old lady who was diagnosed as Hb SS by cellulose acetate alkaline electrophoresis during childhood, but whose parents were known Hb AS and Hb AA respectively, using the same method. This remained a mystery in the family until the lady presented herself at age the age of 28 years to one of the authors and she was investigated. The results showed packed cell volume of 28.6%, low mean cell volume (MCV-65.6fl), mean corpuscular haemoglobin (MCH-23.2pg), mean cell haemoglobin concentration (MCHC-31.0g/dl) while haemoglobin profile also showed high Hb A_2 (4.7%), Hb A (7.7%), Hb S (73.1%) and Hb F (14.5%) and she was diagnosed as Hb Sbeta-thalassemia+. The parents were also invited and diagnosis of beta-thalassemia trait was made in the mother.

Conclusion:
This report shows that the routine cellulose acetate paper in alkaline medium used in determining the Hb phenotype is not adequate to make accurate diagnosis of sickle cell disease especially when it is co-inherited with beta-thalassaemia. We recommend that HPLC should be made readily available at very low cost in this environment.

Keywords: beta-thalassemia, haemoglobin phenotype, mean cell volume, haemoglobin A_2

INTRODUCTION

Haemoglobinopathies are a group of inherited genetic disorder and is the most common single-gene defect in man.[1] The disorder can either be quantitative or qualitative, the quantitative disorders are called thalassaemias which results from decrease or absent production of globin chain while the qualitative disorder is the variation in amino acid arrangement/sequence of the globin chain.[2] The qualitative assessment of haemoglobin type with focus on Hb A, S and C are commonly done in this environment. The methods of determining the Hb genotype requires DNA Analysis using molecular techniques which are not readily available in developing countries like Nigeria. The routine diagnostic method in this environment is the use of cellulose acetate paper in alkaline medium (pH of 8.4) in which the migration of Hb S, D and G are similar while the Hb C, E, A2 and O have similar migrations thereby limiting the accuracy of the diagnosis to only phenotypic assessment. The standard method of assay for haemoglobin variants quantitatively is the use of HPLC which is not readily available in developing countries like Nigeria. A report by Esan in 1970 confirms that 0.8% of Nigerian population have beta-thalassemia trait which was not in agreement with the 9% reported in Liberia despite the similarity in climate and malaria endemicity.[3-5] This report evaluates a case of a 28 year old lady who was diagnosed as Hb SS by cellulose acetate alkaline electrophoresis (pH-8.4) at age of 12 years whose parents were known Hb AS and Hb AA using the same methods.

CASE REPORT

We report a case of a 28 year old lady who was a known Hb SS using cellulose acetate alkaline electrophoresis (pH-8.4) and had blood transfusion at age of 12 years when she had severe anaemia and was transfused with 2 pints of whole blood. The parents were already known to have Hb AA and Hb AS using the same cellulose acetate electrophoresis method at alkaline pH. This remains a mystery in the family until the lady presented herself at the age of 28 years to one of the authors. There was history of bone pain crisis, yellowness of eyes, severe anaemia and blood transfusion. General physical examination showed a young lady, healthy looking, oriented in time, place and person, not in obvious respiratory distress, mildly pale and jaundiced, acyanosed, no abdominal tenderness with no Organomegaly. Five millilitres of blood sample were collected into an EDTA bottle for full blood count and haemoglobin profiling using automated haematological analyzer (ABX Pentra XL 80 – Horiba Medical) and high performance liquid chromatography (HPLC- Bio rad D-10) respectively. The results of the investigations showed a packed cell volume (PCV 28.6%), low mean cell volume (MCV-65.6fl), mean corpuscular haemoglobin (MCH-23.2pg), mean cell haemoglobin concentration (MCHC-31.0g/dl) while haemoglobin profile also showed raised Hb A_2 (4.7%), Hb A (7.7%), Hb S (73.1%) and Hb F (14.5%) as shown in Table 1. The diagnosis of Hb S-beta-thalassemia (Sβ+thal) was made based on the above results. The parents were invited, counselled, and asked to do full blood count and haemoglobin profiling. The results of the mother showed the PCV of 36.2%, low MCV of 69.8 fl, MCH of 20.7pg, MCHC of 29.7 g/dl, Hb F of 1.2%, Hb A of 94.0%, Hb A_2 of 4.8% (Table 1). The diagnosis of beta-thalassemia trait was made based on low MCV, high Hb A_2

and normal Hb A. The results of the father showed the PCV of 37.9%, MCV of 92.4fl, MCH of 29.4pg, MCHC of 31.8g/dl, Hb A of 58.4%, Hb S of 37.3%, Hb A_2 of 3.3%, Hb F of 1.0% (Table 1). These results show Hb AS.

DISCUSSION

Haemoglobin structure with α2 β2 is the normal adult Hb known as Hb A that accounts for 97% of total Hb.[6] Other Hb types present in adult are Hb A2 ($\alpha_2\delta_2$) which accounts for about 2% and Hb F ($\alpha_2\gamma_2$) accounts for about 1% of adult haemoglobin.[7] Beta-thalassemia is one of the major inherited haemoglobin disorders, which is usually misdiagnosed in developing countries like Nigeria especially when presenting with major symptoms like chronic anaemia.[8] The diagnosis of beta-thalassemia is often missed in Nigeria because of the method used in determining the haemoglobin abnormalities. The co-inheritance of Hb S and beta-thalassemia trait has been reported among Nigerians with sickle cell anaemia.[3,8] Kotila and colleagues in 2009 reported the co-existence of Hb A and beta-thalassemia in Nigeria among 50 participants with Hb A type using the level of Hb F and Hb A2.

Table 1: Full blood counts and haemoglobin profiles of the patient and the parents

Parameters	Patient	Mother	Father
PCV (%)	28.6*	36.2	37.9
MCV (fL)	65.6*	69.8*	92.4
MCH (pg)	23.2*	20.7*	29.4
MCHC (g/dl)	31.0*	29.7*	31.8
Hb F (%)	14.5$^+$	1.2	1.0
Hb A_2 (%)	7.7$^+$	4.8$^+$	3.3
Hb A (%)	4.7*	94.0	58.4
Hb S (%)	73.1	-	37.3

***Reduced below normal range $^+$Increased above normal range
PCV- packed cell volume, MCV- mean cell volume, MCH- mean corpuscular haemoglobin, MCHC- mean cell haemoglobin concentration

The Hb A_2 level was determined by elution after electrophoresis on cellulose acetate paper.[9] Inusa and colleagues in 2015 reported prevalence of Hb S and beta (β)-thalassemia co-inheritance in infants of the northern region of Nigeria to be 1% using beta gene sequencing and/or PCR analysis.[12]

In this index report, we used HPLC to quantify the level of Hb A_2, which is known to be more accurate than electrophoretic method in determining Hb A_2 and Hb F levels.[3, 6] Beta-thalassemia trait can be suspected in an individual with low MCV and mean corpuscular haemoglobin (MCH), which will necessitate the quantification of Hb A_2 using the standard

method HPLC.[10] The low level of MCV and high level of Hb A_2 were used in the index case as it has been used in other studies. Daniel and colleagues in 2011 emphasised the need to initiate routine FBC analysis and testing parental samples as a cost-effective means of confirming suspected cases.[11] The index case confirmed this assertion as evident by inheritance of the sickle cell gene from the father and beta-thalassaemia gene from the mother.

CONCLUSION

The routine cellulose acetate paper Hb electrophoresis in alkaline medium is not adequate for Hb phenotype determination for our clinical practice; HPLC should be made readily available at a very low cost to exclude concomitant beta-thalassemia inheritance.

Limitation:
Inability to carry out DNA analysis.

Consent:
Written informed consent was obtained from the patient for publication of this report and any accompanying results.

Availability of data and materials
Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Abbreviations
Hb: Haemoglobin; HPLC: high performance liquid chromatography; MCH: mean corpuscular haemoglobin; MCHC: mean cell haemoglobin concentration; MCV: mean cell volume

Conflict of Interest:
The authors declare that they have no competing interests.

Authors' Contribution:
MAA, RAB and OJO carried out the medical screening and drafted the manuscript. ASO and OO were involved in revising it critically for intellectual content. All the authors read and approved the final manuscript.

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