Case Study

Rare double heterozygous of HbD/HbG in a Nigerian: A case report

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Abstract

Aim: To advocate the use of newer and improved methods towards accurate diagnosis of haemoglobinopathies

Case presentation: A rare case of double heterozygous of HbD/G in a pregnant female Nigerian who had present to the antenatal clinic for routine Haemoglobin electrophoresis. She had previously been diagnosed as HbAS using capillary electrophoresis and HPLC techniques.

Discussion: Capillary zone electrophoretograms showed the presence of peaks in zone Hb A, Hb D, C and a small peak in Z1 zone. Bio-Rad D10 chromatogram also indicated the presence of four peaks which are identified as Hb A, Hb D, Hb G, and hybrid of HbD/HbG. A peak in Hb D zone of capillary electrophoresis was due to co-migration of Hb D and Hb G variants. The small peak in Z1 zone indicated the presence of alpha chain variant of HbG.

Conclusion: The case exemplifies the need to use more advanced methods, including DNA analysis in order to accurately diagnose haemoglobinopathies in the nation with the largest burden of sickle cell disease.

Keywords: Haemoglobinopathies, haemoglobin electrophoresis, heterozygous, high performance liquid chromatography

Introduction

Haemoglobinopathies are a group of genetic disorders that lead to quantitative or qualitative abnormalities in haemoglobin (Hb) variants. Thalassaemias are due to the reduced or absent production of structurally normal globin chains while sickle cell disease occurs because of substitution of one amino acid by another. The change in amino acid sequence results in haemoglobin variants with abnormal structures. Many of the haemoglobin variants do not cause symptoms in heterozygous condition but can lead to varying degrees of anaemia and other symptoms in homozygous states or when they coexist with thalassaemias.

Double heterozygosity is described when there is a change in the amino acid sequence in both α and β chains of the same individual and it is very rare. Though, sporadic cases of hybrid haemoglobins have been reported in other regions of the world, here we report the very first case of double heterozygosity of an alpha-chain variant hemoglobin G and a beta chain hemoglobin D in a Nigerian.

Case Report

A 28 year old female, Yoruba by tribe from the Southwestern region of Nigeria presented to the laboratory for Haemoglobin electrophoresis as part of the routine antenatal investigation. She has no significant past medical history. Her previous Haemoglobin electrophoresis (verbal report from patient) by cellulose acetate at alkaline pH was reported as HbAS. About 3mls of venous blood was collected into an ethylenediamine tetraacetic acid (EDTA) bottle. Complete blood count and solubility tests were also carried out on the sample.

Capillary zone electrophoresis (CE) for the sample was carried out using automated Sebia Minicap analyser (Sebia, France) according to the manufacturer's instructions and was repeated with BIORAD D10 high performance liquid chromatography (HPLC) to further identify and confirm the results. The electrophoretogram by Sebia Minicap analyser showed four main peaks as follows: HbA zone (53.5%), HbD zone (22.4%), HbA2 zone (5.1%) which slightly overlaps with an unknown peak in C zone (15.3%). There was also a small peak in the Z1 (2.5%) indicating a variant of alpha chain (fig 1).

The BIORAD-D10 high performance liquid chromatography also showed four major peaks HbA (32.5%) with retention time of 1.69 minutes, HbD (30.4%) with a retention time of 3.91minutes in an unknown window, HbG (17.1%) with retention time 4.09 minutes in Hb S-window, and an hybrid of HbD/G (12.7%) at retention time of 4.41 minutes in an unknown window (fig 2). A small peak of HbA2 (2%) is also noted on the electrophoretogram. The full blood count showed essentially normal parameters: RBC= 4.3 X 10^6/μL, Heamoglobin= 12.5g/dl, mean cell corpuscular volume (MCV)=88FL, mean corpuscular haemoglobin concentration (MCHC)=33g/dl, mean corpuscular haemoglobin (MCH)= 29pg The sickling test, a procedure in which red blood cells sickle in the presence of sodium metabisulphite (a reducing agent) was negative, however, solubility test was not done. The requesting

physician was advised of the need for DNA analysis to confirm the diagnosis. However, patient was lost to follow up.

Figure 1: Capillary zone electrophoresis pattern indicates peaks in HbA, Hb D, Hb C, HbA2 zones, and additional small peak in Z1 zone indicating alpha chain variant

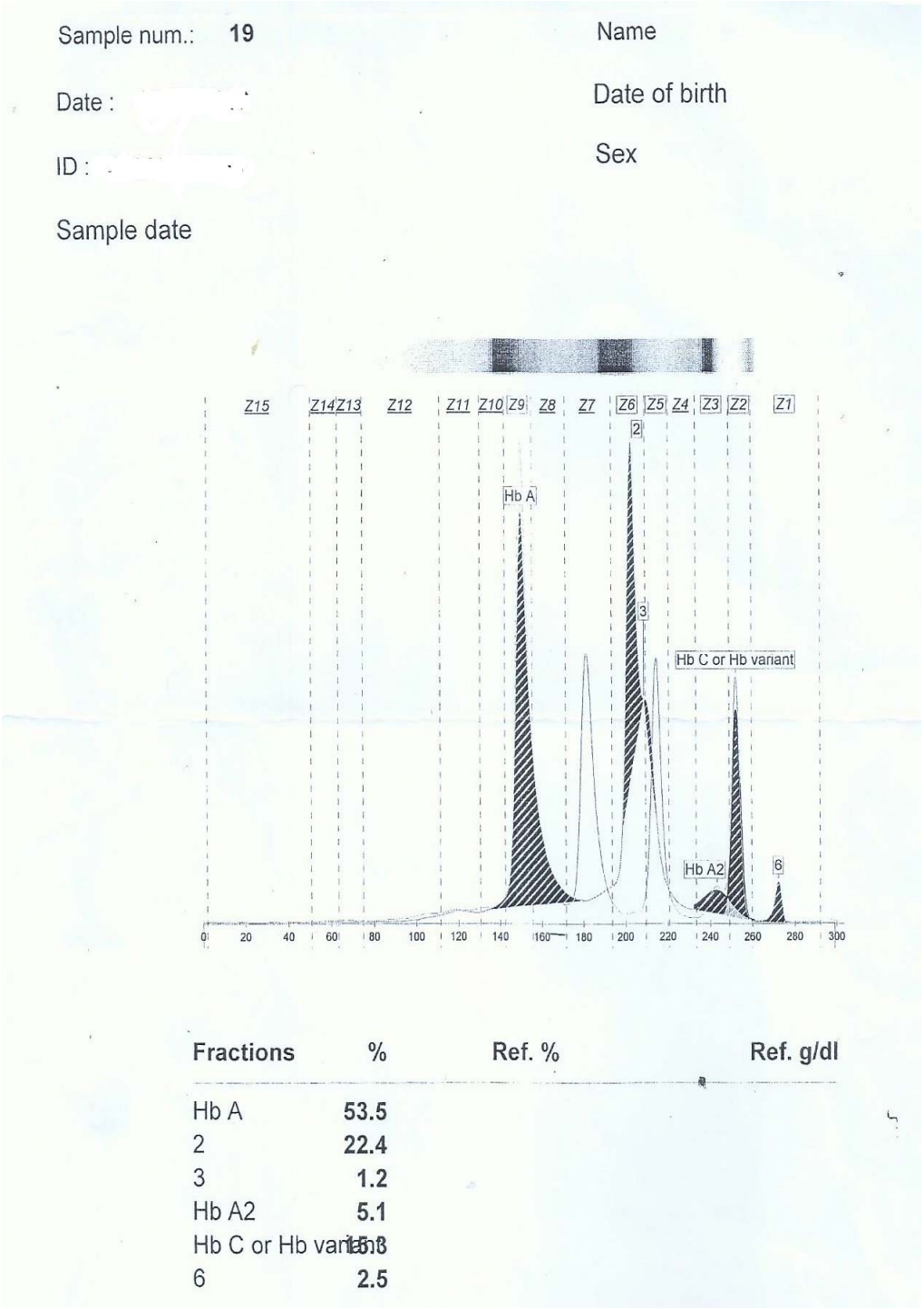
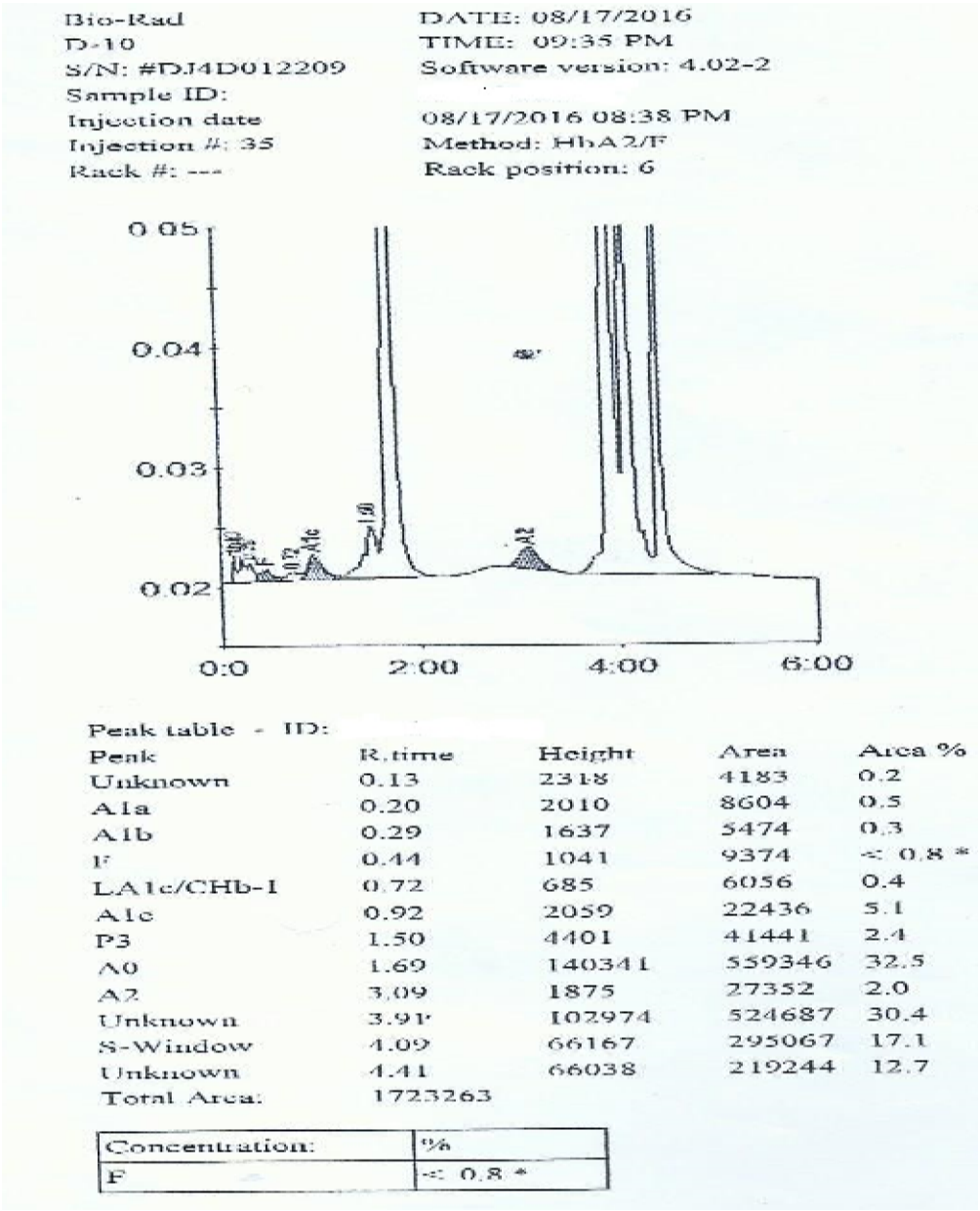


Figure 2: High performance liquid chromatography (HPLC) obtained in BIORAD D10 showing 4 major peaks at retention times in minutes 1.69 (HbA), 3.91 (HbD), 4.09 (HbG), 4.41 (hybrid of HbD/G)



Discussion

There are a few variants of haemoglobin D. Hb D Punjab or Hb D Los Angeles is a type of beta globin gene mutation at 121 codon resulting in replacement of glutamic acid with glutamine (Glu->Gln).[1] The highest prevalence of HbD Punjab is among Sikhs in Punjab, India where it is reported to be around 2%. Heterozygous HbD is a clinically silent condition.[1] There is also HbD Ibadan which was discovered at the University College Hospital Ibadan, Nigeria.[2] The prevalence of this is currently unknown. Hb D Ibadan results from the replacement of Threonine with Lysine in position 87 of beta chain (Threonine> Lysine).[2] Haemoglobin G Philadelphia is the most common alpha chain variant and is due to replacement of asparagine with Lysine ($\alpha^{68\ Asn>Lys}$).[3] It occurs in less than 1% of the population of West Africa.[4]

Presumptive identification of Hb variants was done by comparing the two methods used. Both techniques clearly indicated that the predominant haemoglobin in this subject was HbA (figs 1 & 2). The second peak on Capillary zone electrophoresis (fig 1) could be either haemoglobin D or G or both travelling within zone 6.[5] Following review of the literature we confirmed that HbD elutes at approximately 3.91minutes in D window similar to what was obtained on our HPLC (fig.2).[7,8] Furthermore, an unknown haemoglobin eluted in the S-window on HPLC at retention time of 4.09 minutes but there was no corresponding pattern in zone 5 (S zone) of CE, suggesting it is unlikely to be HbS but rather HbG which co-eluted with HbD in zone 6 of capillary electrophoresis. Haemoglobins S, D and G also have the same mobility on cellulose acetate paper at alkaline pH which explains the initial diagnosis of HbAS as claimed by the patient. Cellulose acetate method is the routine technique in most laboratories in Nigeria. The unknown pattern in the C window on Capillary electrophoresis is the hybrid of HbD/G (fig 1). This correlates with the unknown peak eluted at 4.41 minutes in the HPLC (fig 2). The inheritance of alpha-chain defects such as HbG-Philadelphia usually results in formation of hybrid haemoglobins.[9] The small peak of 2.1% in Z1 zone highly suggests the presence of alpha variant of HbG The haematological parameters and indices were normal in this patient. The patient had no clinical symptoms and had only presented to the hospital for antenatal booking. Therefore, the clinical implications of this inheritance cannot be determined at the moment. The limitation of this study is non-availability of facilities to further confirm the identity of the various haemoglobins in this patient. DNA sequencing of alpha and beta globin genes which is the confirmatory diagnostic method is not readily available in Nigeria. The traditional method of haemoglobin electrophoresis using cellulose acetate in alkaline pH will most probably misdiagnose this patient.

The fully automated methods such as HPLC and CE have replaced the cellulose acetate electrophoresis as first-line in the diagnosis of haemoglobinopathies. Apart from the advantages of resolution and automation both allow processing of large batches and require very small samples volumes.[10,11,12] Quantification and identification of larger proportion of variant haemoglobins can be made. The major disadvantage of HPLC is that it separates gycosylated and other derivatives of haemoglobin making its interpretation complex, however this does not occur with CE. CE has also been found to be more accurate and sensitive for detecting Hb variants than cellulose acetate paper.[10,13]

Conclusion: This case exemplifies the relevance of advanced methods such as DNA techniques in diagnosis of haemoglobinopathies. Quantitative haemoglobin electrophoresis techniques such as Capillary electrophoresis and HPLC have been recently available in some diagnostic laboratories in Nigeria, although not so affordable to the general population.

However, DNA analysis that would have helped in making definitive diagnosis in the index case, is still not available.

Consent: Consent obtained from the patient.

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