Case Report

Hemoglobin Fontainebleau [a21(B2)Ala>Pro]: The second report from India

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Structural hemoglobin (Hb) variants are mainly due to point mutations in the globin genes resulting in single amino acid substitutions. Until date, about 200 alpha chain variants have been identified and they are usually detected during the hemoglobinopathy screening programs. Under a community control program for hemoglobinopathies, which involved screening of antenatal cases followed by prenatal diagnosis if indicated. Here, we report a rare alpha globin gene variant Hb Fontainebleau [a21(B2)Ala>Pro] detected in the heterozygous condition in a 35-year-old pregnant lady screened during this program. This is the second report of this alpha globin variant from India. Unlike the earlier case from India where Hb Fontainebleau was reported in a neonate who was also a carrier of Hb Sickle and had no clinical problems, this case presented with a bad obstetric history associated with the secondary infertility. However, the presence of the variant and the obstetric complications may be unrelated.

Key words: Alpha globin gene variant, hemoglobin Fontainebleau, infertility

Introduction

Structural hemoglobin (Hb) variants are mainly due to point mutations in α or β globin genes resulting in single amino acid substitutions. Until date, around 1000 Hb variants have been identified out of which at least 200 are alpha chain variants.[1] Many of these variants do not cause any problems and therefore remain undetected in the population. They are often picked up only when a large screening program on hemoglobinopathies is undertaken in the general population. Few alpha chain variants like Hb Sallanches, though asymptomatic in the heterozygous condition cause severe Hb H disease in the homozygous condition.[2] Until date, four cases of Hb Fontainebleau have been reported, including one case from India.[3-6] These have all been present in combination with Hb S or a co-existing membrane defect. We report the first report of Hb Fontainebleau from India without any other co-existing hematological defect.

Christian Medical College and Hospital, Ludhiana is a tertiary care center. During the Jai Vigyan Mission Project on screening and community control of thalassemia and sickle cell disease by Indian Council of Medical Research (ICMR) from 2001 to 2004, 5000 antenatal cases (ANC) were screened, of which 4.2% were detected to have heterozygous state for beta thalassemia and 1.8% consistent with Hb D-Punjab, Hb D-Iran, Hb Q-India, Hb E and Hb S trait. Subsequently, antenatal screening continued in our institute as Phase II of the above project. An ICMR sponsored center for molecular characterization of hemoglobinopathies and prenatal diagnosis of thalassemias and sickle cell disease was established in our institute.

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Recently, a 35-year-old ANC born of a non-consanguineous marriage to a Jat Sikh family from Punjab was presented for routine thalassemia screening

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under the antenatal screening program for thalassemia. She had no previous history of anemia or blood transfusions. She had no clinical complications other than a bad obstetric history. She had delivered a healthy baby boy at 26 years of age by a cesarean section with a low birth weight of 1.7 kg. This pregnancy was associated with pregnancy induced hypertension, oligohydramnios and intrauterine growth retardation. She had secondary infertility and later conceived on two occasions at the age of 33 and 34 years. These were high risk pregnancies associated with hypothyroidism and ended in a missed abortion at 9 weeks gestation and medical termination of pregnancy at 20 weeks gestation, respectively. No definitive cause of infertility could be ascertained.

Her Hb level was 11.9 g/dl with red blood cells (RBCs) count of 4.37 million/μl, mean cell Hb of 27.2 pg, mean cell volume of 81.9 fl and red cell distribution width 16.0%. RBCs were normocytic normochromic. High-performance liquid chromatography (HPLC) variant study showed an unknown peak of 14.9% (retention time 2.89 min), which appeared as a hump in the peak adjoining Hb A [Figure 1]. Cellulose acetate electrophoresis (pH 8.9) did not show the presence of any abnormal band. The common alpha globin gene deletions were found to be absent. Direct deoxyribonucleic acid (DNA) sequencing of the alpha globin gene showed the presence of a heterozygous G→C substitution at codon 21 leading to the substitution of alanine to proline corresponding to Hb Fontainebleau [Figure 2]. The detailed hematological and molecular findings are shown in Table 1. Her husband was also screened for hemoglobinopathies and showed normal red cell indices and a normal Hb chromatogram on HPLC. Other family members were not available for screening. The couple was suitably counseled.

Discussion

The spectrum and frequency of α globin chain variants in India remains unknown though few cases have been reported.[7] Non-deletional mutations, in general, affect the fundamental processes of globin gene expression. Hb Fontainebleau [a21(B2)Ala>Pro], is an alpha chain variant characterized by an alanine→proline substitution at codon 21 with a GCT→CCT change at the DNA level,

Table 1: Hematological and molecular investigations in the case heterozygote for Hb Fontainebleau

Parameter	Case
Age (year)/sex	35/F
Caste and community	Jat Sikh
Origin	Punjab
RBC (×10^6/μl)	4.37
Hb (g/dl)	11.9
MCV (fl)	81.9
MCH (pg)	27.2
RDW (%)	16.0
HbA2 (%)	2.8
Hb F (%)	0.0
Hb A (%)	72.4
Unknown Hb% (on HPLC)	14.9 (retention time 2.89 min)
Cellulose acetate electrophoresis (pH 8.9)	Normal
α gene mutation	CD 21 (GCT→CCT) Ala→Pro

Hb: Hemoglobin, HPLC: High-performance liquid chromatography, RDW: Red cell distribution width, MCH: Mean cell hemoglobin, MCV: Mean cell volume, RBC: Red blood cell

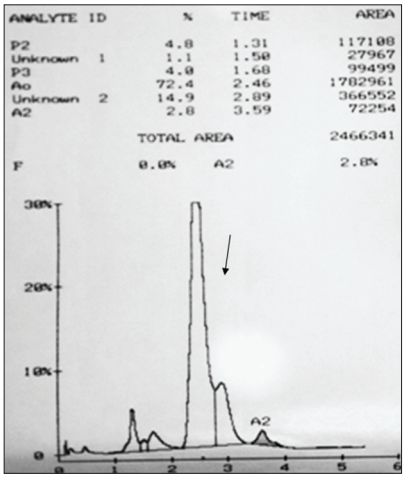

Figure 1: High-performance liquid chromatography chromatogram showing the unknown peak at retention time - 2.89 min

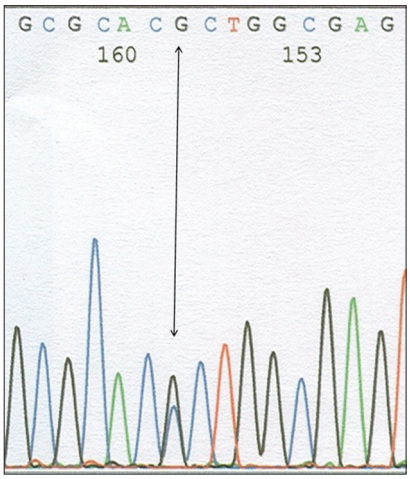

Figure 2: Electropherogram of the alpha gene showing hemoglobin Fontainebleau [a21(B2)Ala>Pro]

this proline residue is located at the beginning of the alpha helix.[3] This variant has so far been identified in only four unrelated families globally. It was first reported in a 15-year-old girl of Italian origin living in France. In this case, the co-existing membrane defect, spherocytosis with Hb Fontainebleau explained the hematological abnormalities and the severe phenotype.[3] Another female individual in the same family with Hb Fontainebleau alone was clinically and hematologically normal. The second case was an adult male subject in an Iraqi family living in New Zealand. This individual had microcytosis, but this was not associated to the presence of Hb Fontainebleau.[4] The third case was identified in Cyprus while screening for thalassemia in the Greek Cypriot population.[5] The fourth family was from Madhya Pradesh, India in a newborn baby and her mother. In both these cases, this mutation was present along with a beta globin gene defect Haemoglobin Sickle Hb S.[6]

This is the second report of this Hb variant from India. Unlike the earlier case, this case had normal hematological indices, but presented with a bad obstetric history, which may just be a chance association.

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