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Asymptomatic Child Heterozygous for Hemoglobin S and Hemoglobin Pôrto Alegre

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Abstract

Hemoglobin Pôrto Alegre (PA) is a rare hemoglobin resulting from a mutation in $\beta 9(A6)Ser \rightarrow Cys$. We describe an asymptomatic Puerto Rican female with combined heterozygosity for Hb PA and Hb S. Since birth, she has maintained normal hemoglobin, bilirubin, LDH levels, and reticulocyte count. Peripheral smear evaluation has revealed normal erythrocyte morphology with no changes suggestive of hemolysis. We conclude that the presence of Hb PA does not increase the risk of red blood cell sickling in patients who carry the Hb S mutation.

Keywords

Anemias; Hematology; Hemoglobinopathies; Hemoglobin Porto Alegre; Sickle Cell Disease

INTRODUCTION

A large number of inherited anomalies of human hemoglobins have been reported since the introduction of electrophoretic techniques into medicine [1]. Hemoglobinopathies are the most common inherited disorders in humans; the most frequent are hemoglobins S and C [2]. Hemoglobin Pôrto Alegre results from a mutation in $\beta 9(A6)Ser \rightarrow Cys$ which induces hemoglobin polymerization by forming interchain disulfide bonds via the extra cysteine [3]. Previous studies report that homozygous and heterozygous Hb PA forms polymers in vitro, but exists as a tetramer in vivo [4]. This may account for the lack of anemia, erythrocyte morphologic abnormalities, or associated clinical symptoms in individuals who are homozygous and heterozygous for this abnormal hemoglobin [4]. As Hemoglobin PA is clinically and electrophoretically a silent mutant [5], it is likely that its presence does not induce red blood cell sickling in persons who are also carriers of the hemoglobin S mutation.

CASE REPORT

A term Puerto Rican infant, born without complications to unrelated parents, was found to have Hb S and an unusual fast moving band by isoelectric focusing electrophoresis during her newborn screening panel done at the Center for Hereditary Diseases at the University Pediatric Hospital in San Juan, Puerto Rico. Mass spectrometry, done at the Haemoglobinopathy Screening Services in St. James University Hospital in Leeds, UK, revealed a molecular weight of 15,883 compatible with Hb PA. Further analysis took place under an approved Institutional Review Board of the University of Puerto Rico Medical

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Sciences Campus. Informed consent was obtained from our patient's parents prior to additional testing.

DNA sequencing was then performed at the Human Molecular RCMI Core Lab in the Medical Sciences Campus, University of Puerto Rico. For PCR and sequencing, DNA was isolated from peripheral leukocytes using the QIAamp DNA Blood Midi kit (Qiagen, CA). DNA fragments were amplified using the standard polymerase chain reaction conditions, using BGPL forward primer 5'-AAGAGCCAAGGACAGGTAC and BGEX1R reverse primer 5'-GGTCTCCTTAAACCTGTCTTG. The PCR products were purified using the Qiaquick gel extraction kit (Qiagen, CA) according to the manufacturer's instructions and were sequenced using an ABI-Prism 310 Genetic Analyzer (Applied Biosystems, USA). The sequence data were examined using Finch TV software version 1.4.0. DNA sequencing confirmed the presence of Hb Pôrto Alegre, with mutation $\beta 9(\text{A6})\text{Ser} \rightarrow \text{Cys}$ as well as the presence of the $\beta 6(\text{A3})\text{Glu} \rightarrow \text{Val}$ mutation. As seen in Figure 1, the TCT codon in Fig. 1A encodes for the expected serine (both alleles) in a control patient, while Fig. 1B shows our patient with TCT/TGT overlap secondary to heterozygosity for Hb PA. The patient's father was found to be heterozygous for Hb S and the mother a carrier for Hb PA.

Our patient has been followed closely for the past three years where she has remained asymptomatic without clinical or hematological evidence of hemolytic disease (Table I). Our patient has been growing and developing well. Physical examination has not revealed tachycardia, pallor, jaundice, or splenomegaly. She has maintained adequate hemoglobin levels. An isolated hemoglobin of 10.2 g/dl, consistent with physiologic anemia of infancy, was documented at 3 months of age. She has maintained normal bilirubin and LDH levels and adequate reticulocyte counts. Repeated peripheral smear evaluation has revealed normal erythrocytic morphology with no changes suggestive of hemolysis.

DISCUSSION

Hemoglobin Pôrto Alegre (PA) is a rare hemoglobin resulting from a mutation in $\beta 9(\text{A6})\text{Ser} \rightarrow \text{Cys}$, which results in high molecular weight oligomers through covalent [disulfide] bonds [3]. The normal electrophoretic mobility of a fresh un-oxidized hemolysate of homozygous patients indicates that the mutation causing Hb PA [does] not alter the net charge of the molecule, and that in vivo the molecule exists as a tetramer [4]. For this reason, the patient's Hb PA migrates in the position of Hb A in electrophoresis.

It has been reported that cells carrying Hb Pôrto Alegre contain higher amounts of glutathione [5]. It has been suggested that the increase in glutathione is responsible for the absence of in vivo polymerization and lack of clinical symptoms [5]. A kinetic study of hemoglobin Porto Alegre has demonstrated that decreasing the initial proportion of GSH in a hemolysate increases the formation of Hg PA disulfide polymers [6]. In sickle cells, glutathione bound to Hb inhibits the polymerization of Hb S by increasing the oxygen affinity of Hb and also by direct inhibition of contacts in the polymer [7]. Therefore, it seems reasonable to propose that increased levels of glutathione in the red cells of our patient would not favor polymerization of either Hb S or Hb PA.

As described in a family of Portuguese origin, Hb PA is a clinically silent hemoglobin variant even in homozygotes [8]. Furthermore, it has been found that Hb PA does not lead to clinical problems when associated to beta thalassemia or Hb Santa Ana because it can coexist with abnormal variants [9]. Hemoglobin Pôrto Alegre has been documented in a few patients from Brazil, Portugal, Argentina, Cuba, and the Canary Islands. The presence of double heterozygosity for Hb S and Hb PA has not been previously reported. Given that our patient has remained asymptomatic and has had normal hematologic parameters, we

conclude that the presence of Hb PA is unlikely to promote red blood cell sickling in carriers of Hg S. Similar to patients with sickle cell trait, patients heterozygous for Hb S and Hb PA would be expected to remain asymptomatic, without evidence of hemolytic disease.

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Fig. 1. Chromatogram generated by sequencing of PCR product from: (A) non-affected control and (B) patient heterozygous (C/G) in position 78 of the beta globin gene reference sequence (gi: 28380636), denoting carrier status for Hb Pôto Alegre.

TABLE I

Laboratory Values Appropriate for Age and Consistent With Lack of Hemolysis

Age	Hb (g/dl)	Hct (%)	Retic (%)	T. bili (mg/dl)	LDH (IU/L)	MCV (fl)	MCH (pg)	MCHC (g/dl)
3 months	10.2	29.7	—	—	—	84.1	28.9	34.3
4 months	11.6	35.0	—	—	—	87.5	28.7	32.8
15 months	11.4	33.0	2.2	0.3	251	73.8	25.4	34.4
36 months	11.8	34.6	1.5	0.6	235	74.1	25.4	34.2

Hb, hemoglobin; Hct, hematocrit; LDH, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; mo, months; Retic, reticulocyte count; T. bili, total bilirubin.