

Case Study

SEVERE HEMOLYTIC ANEMIA DUE TO *de novo* HEMOGLOBIN SABINE IN AN ARGENTINIAN NEWBORN. FIRST CASE IN SOUTH AMERICA

Abstract

Hemoglobin (Hb) Sabine is an unstable Hb variant that causes hemolytic anemia in heterozygous state, with inclusion bodies in the red blood cells (RBC). This hemoglobin is the result of a point mutation at codon 91(CTG)→(CCG) of the beta-globin gene. We report, for the first time in South America, the identification of Hb Sabine in a nine-month-old female baby, referred to our laboratory bearing a severe hemolytic anemia. We emphasize the need for the correct characterization of this unstable hemoglobin mainly for therapeutic purposes and for genetic counseling.

Key words: Abnormal hemoglobin (Hb), Hb Sabine, Unstable hemoglobin.

Introduction

Up to date, there have been described more than 140 unstable hemoglobins (1), related to hemolytic anemia with different clinical expression. The mutations that cause destabilization of the tetrameric structure are the most frequent cause of hemolytic anemia. Proline introduction in the alpha helix, beyond the third residue, distorts the hemoglobin structure and cause instability. In general, these undergo increased oxidation of the heme with the formation of metHb and further degradation leading to precipitated Hb and other products within the circulating red cells. (2) In Hb Sabine, as in many of the other unstable hemoglobins, the substitution is a neutral one [β 91(F7)Leu→Pro], so no alteration in charge of the globin chain would be expected. The diminished anodal mobility of these hemoglobins is probably due to loss of heme, an event to which they are peculiarly prone.(3) Hb Sabine is an unstable β chain variant which causes moderately severe hemolytic anemia and has been reported in a few unrelated patients, none of them from South America till date (4-8). In this paper, we report a new *de novo* case of Hb Sabine in a nine-month-old female baby. The mutation was identified by DNA sequencing following amplification by polymerase chain reaction (PCR). The proband's father is of German origin and her mother is of Spanish descent. The girl has no familial history of anemia. Paternity was confirmed through studies of DNA polymorphism (STRs loci).

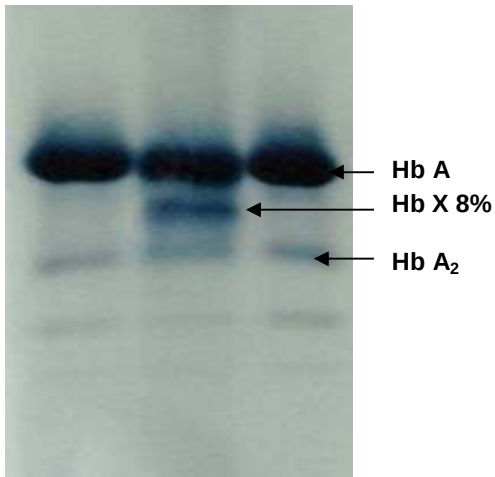
Case report

The child was admitted to the Service of Pediatrics because of a moderately severe anemia, pallor and a subicteric condition. Her facies indicates expansion of haemopoietic tissue in the skull bones, particularly in the frontal and parietal bones. She had never been transfused. At the age of seven months, she suffered an hemolytic episode in the course of an infection.

Hematological data were obtained with a Sysmex KX21 blood counter. The blood film showed microcytosis, polychromasia, target cells, coarse basophilic stippling, and 2 nucleated erythroid progenitors per 100 white cells. The Hb A2

51 was measured by elution post electrophoresis at alkaline pH, and Hb F
52 according to the method described by *Betke et al.*(9)
53 Isopropanol test (*Carrell & Kay*) (10) was performed. Cellulose acetate
54 electrophoresis at alkaline pH and globin chains electrophoresis at alkaline pH,
55 were carried out using standard methods.
56 Sickling test was negative. The isopropanol test was positive indicating the
57 presence of an unstable Hb. Cellulose acetate (pH 8.4) electrophoresis
58 detected an additional Hb fraction, between Hb A and Hb A₂. (Figure 1) Globin
59 chains electrophoresis did not show the presence of an abnormal chain.

60
61 Figure 1
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68 Hemoglobin electrophoresis at alkaline pH.
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71 The patient's hematological and biochemical data are shown in Table 1 and
72 hematological and hemoglobin composition data of parents are shown in Table
73 2.
74
75

76 Table 1

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parameters	proband
Hb A/X/A2 (%)	8
Hb (g/L)	770
RBC ($10^{12}/L$)	2.84
MCV (fL)	95.1
MCH (pg)	27.1
Reticulocytes (%)	40
A2 (%)	2.6
Hb F (%)	10
Isopropanol Test	positive
Sickling Test	negative
Total serum bilirrubina (umol/L)	35.2 (NR:5.7-17)
LDH U/l	1075 (NR:360-720)
Ferritina (ug/dl)	84 (NR:24-120)
Iron (ug/dl)	114 (80-110)
TIBC (ug/dl)	351 (280-330)

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80 Hematological and biochemical data of the patient

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82

83 Table 2

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Subjet	Hb g/L	RBC ($10^{12}/L$)	MCV (fL)	MCH (pg)	Reticulocytes (%)	A2 (%)	Hb F (%)	Hb X (%)
Father	152	4.8	91.7	31.7	1.3	2.4	1	0
Mother	134	4.27	88	31.4	1.2	2.6	0.9	0

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87 Hematological and hemoglobin composition data of parents

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89 DNA was extracted from peripheral blood samples as previously described (11).
 90 Polymerase chain reaction (PCR) was carried out and then the coding regions
 91 of the β - and α -globin genes were sequenced. It was performed using a Big Dye
 92 Terminators Ready Reaction Kit (Perkin-Elmer Cetus, Norwalk, CT, USA) in an
 93 ABI PRISM 310 sequencer (Perkin-Elmer Cetus).

94 Alpha1 and α 2 genes were normal, while sequencing of β gene revealed a
 95 CTG→CCG (Asp→His) substitution at codon 91, corresponding to Hb Sabine
 96 (Figure 2). DNA sequence analysis of the β -globin gene of both parents showed
 97 the absence of the Hb Sabine mutation. Paternity was confirmed by the study of
 98 nine short tandem repeats (STRs) and four variable-number tandem repeat
 99 (VNTRs) loci.

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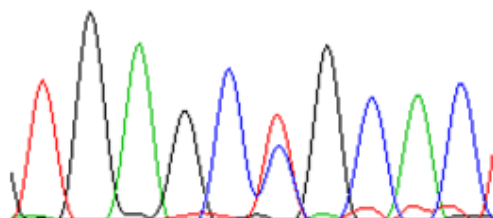
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103

104 Figure 2

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T G A G C N G C A C



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107 Sequence of the β -globin gene: CTG→ CCG (Asp→His) substitution at codon
108 91 corresponding to Hb Sabine.
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110 Discussion

111 Numerous cases of hemolytic anemia have been described that are a
112 consequence of the presence in the erythrocyte of unstable hemoglobins.
113 Most unstable hemoglobin variants in heterozygous state have a variable
114 percentage of the variant. In our case it was 8% of total hemoglobin. This is a
115 much smaller proportion than that found for most Hb variants, presumably
116 because it is precipitated both *in vivo* and *in vitro* during manipulation in the
117 experimental procedures. The erythrocyte containing Hb Sabine, have a
118 markedly shortened survival as a consequence of impaired metabolism,
119 probably due to dissociation and precipitation of abnormal beta polypeptide
120 chains of Hb Sabine. Unstable Hbs are inherited in an autosomal dominant
121 fashion, and virtually all affected individuals are heterozygous. The homozygous
122 state for the unstable variants has not been found.

123 Our patient, as all Hb Sabine carriers reported so far had significantly increased
124 Hb F levels (HbF:10%) Patients with higher levels of HbF as those referred by
125 Pavlovic S, et al. (8) and Panagoula Kollia, et al. (15) one had an association
126 with the Xmn I polymorphism at -158 G γ and the other a compound
127 heterozygosity with non-deletional hereditary persistence of fetal hemoglobin
128 respectively.

129 Almost all reported patients suffered from severe hemolytic anemia and were
130 splenectomized at early age.

131 Our patient that now is 17 months is been managed with folic acid and when
132 she was 14 months received one transfusion. Splenectomy can not be
133 predicted now, it will depend on the transfusion regimen, hypersplenism and
134 how she passes her early childhood. In Pediatrics is ideal to splenectomize after
135 the age of 6.

136 Even though Hb Sabine is detectable by electrophoresis, its correct
137 characterization requires more sophisticated methods, like high performance
138 liquid chromatography, capillary electrophoresis or molecular analysis.

139 The so far published cases of Hb Sabine in 1969 (4), 1983 (5), 1992 (6) and
140 2008 (12) were all de novo presentations. The only inherited case reported in
141 2004, was the son of a Yugoslavian patient (8).

Conclusion

We report this case to emphasize the need for the correct identification of this unstable hemoglobin, mainly for therapeutic purposes and for genetic counseling, especially in therapeutic cases (13-14).

These patients present mild to severe chronic hemolytic anemia that may be exacerbated by stress, especially infections, and treatment with oxidizing agents.

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