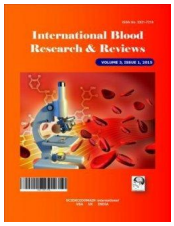

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Severe Hemolytic Anemia Due to *De novo* Hemoglobin Sabine in an Argentinian Newborn. First Case in South America

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SP and IB designed the study, wrote the protocol, wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. Authors MR, MO, LV, IA and AP performed laboratory tests. Authors AP, GD, SZ and MEV managed the literature searches.

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Case Study

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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.

Keywords: Abnormal hemoglobin (Hb); Hb sabine; unstable hemoglobin.

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1. 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 met Hb 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 [β91(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).

1.1 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 a 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 was measured by elution post electrophoresis at alkaline pH, and Hb F according to the method described by Betke et al. [9].

Isopropanol test (Carrell & Kay) [10] was performed. Cellulose acetate electrophoresis at alkaline pH and globin chains electrophoresis at alkaline pH were carried out using standard methods.

Sickling test was negative. The isopropanol test was positive indicating the presence of an unstable Hb. Cellulose acetate (pH 8.4) electrophoresis detected an additional Hb fraction, between Hb A and Hb A2. (Fig. 1) Globin chains electrophoresis did not show the presence of an abnormal chain.

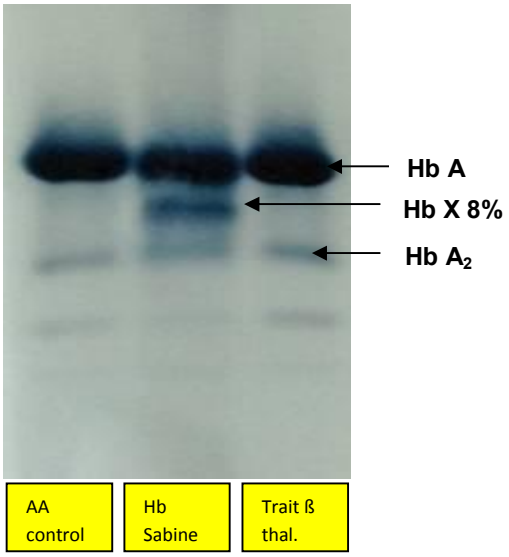

Fig. 1. Hemoglobin electrophoresis at alkaline pH

The patient's hematological and biochemical data are shown in Table 1 and hematological and hemoglobin composition data of parents are shown in Table 2.

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

Alpha1 and α2 genes were normal, while sequencing of β gene revealed a CTG→ CCG (Asp→His) substitution at codon 91, corresponding to Hb Sabine (Fig. 2). DNA sequence analysis of the ß-globin gene of both parents showed the absence of the Hb Sabine mutation. Paternity was confirmed by the study of nine short tandem repeats (STRs) and four variable-number tandem repeat (VNTRs) loci.

Table 1. Hematological and biochemical data of the patient

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)

Table 2. Hematological and hemoglobin composition data of parents

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

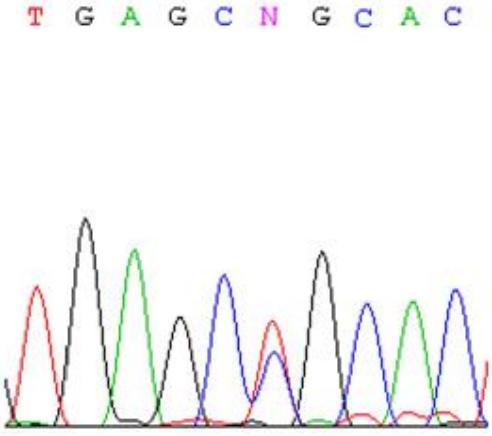

Fig. 2. Sequence of the β-globin gene: CTG→ CCG (Asp→His) substitution at codon 91 corresponding to Hb Sabine

2. DISCUSSION

Numerous cases of hemolytic anemia have been described that are a consequence of the presence in the erythrocyte of unstable hemoglobins.

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

Our patient, as all Hb Sabine carriers reported so far had significantly increased Hb F levels (HbF:10%) Patients with higher levels of HbF as those refered by Pavlovic S el al. [8] and Panagoula Kollia et al. [12] one had an association with the Xmn I polymorphism at -158 Gγ and the other a compound heterozigocity with non-deletional hereditary persistence of fetal hemoglobin respectively.

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

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

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

The so far published cases of Hb Sabine in 1969 [4], 1983 [5], 1992 [6] and 2008 [13] were all de novo presentations. The only inherited case reported in 2004, was the son of a Yugoslavian patient [8].

3. 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 [14-15].

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

CONSENT

All authors declare that written informed consent was obtained from the patient's parents for publication of this case report.

ETHICAL APPROVAL

All authors hereby declare that all laboratoty tests have been examined and approved by the "Comité de Etica de la Facultad de Ciencias Bioquímicas" and have therefore been performed in accordance with the ethical standads laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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