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Hb M-Saskatoon: An unusual cause of cyanosis in a Spanish child

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ABSTRACT

Pulse oximetry in a 15-month-old boy with cyanosis revealed low oxygen saturation (SpO2 60%) with normal echocardiography findings. Arterial blood gas analysis showed normal PaO2, low hemoglobin oxygen saturation, and high methemoglobin level. Blood analysis revealed mild hemolytic anemia. HPLC and electrophoresis showed a variant hemoglobin in the A2-window and C/E band respectively. Beta-globin gene sequencing revealed heterozygous hemoglobin M Saskatoon [Beta63 (E7) His > Tyr; HBB: c 190C > T] along with alpha + heterozygous thalassemia. Hemoglobins M are structural variants that stabilize heme iron in the oxidized (ferric) state. They can be confused with other causes of methemoglobinemia like genetic alterations in methemoglobin reductase enzyme systems of red cells. The prognosis is excellent in these non-life-threatening conditions.

1. Introduction

Methemoglobinemia is a clinical syndrome caused by an increase in the percentage of hemoglobin (Hb) in which heme iron is in the oxidized ferric state (Fe^{3+}) inside of the ferrous state (Fe^{2+}). It can be acquired (drugs, toxins) or congenital, either by genetic defect in red blood cell metabolism (NADH cytochrome b5 reductase deficiency) or through a structural variant of hemoglobin, hemoglobin M (HbM) [1].

We report a 15-month-old boy with HbM Saskatoon associated with α^+ thalassemia diagnosed after the observation of low transcutaneous oxygen saturations measured by pulse oximetry (SpO_2).

2. Clinical case

A 15-month-old boy was referred to our hospital for evaluation in the cardiology department. He was born to non-consanguineous Spanish parents. The patient was asymptomatic except for cyanosis that was first noted at 6–8 months by his parents, after a bout of mild bronchiolitis, and persisting thereafter. He led a normal life without limitations and had normal psychomotor skills for his age. He was receiving treatment with montelukast (a leukotriene receptor antagonist) and inhaled β_2-agonists plus corticosteroids, no other drugs. An arterial blood gas analysis showed normal pH, PaO_2, and pCO_2 values (7.40, 105 mmHg, and 32 mmHg), low hemoglobin oxygen saturation (82%), and high methemoglobinemia (8.5%). Echocardiography ruled out any alterations. Pulse oximetry showed low SpO2 (60%) which did not increase with oxygen administration and central pseudo-cyanosis (brown coloration) with no signs of respiratory distress or other alterations (Fig. 1). A blood analysis revealed the following values: hemoglobin (Hb), 10.9 g/dL; mean corpuscular volume, 81.9 fL; total reticulocytes, 112 000/μL; and red cell morphology slight hypochromic. The remaining blood and iron values were unremarkable. The initial evaluation was based on high-performance liquid chromatography (VARIANT™; Bio-Rad Laboratories, Hercules, CA, USA). The relative concentrations of

Abbreviations: Hb, hemoglobin; HbM, the hemoglobin M; SpO2, pulse oximetry; NADPH, nicotinamide adenine dinucleotide; MetHb, methemoglobin.

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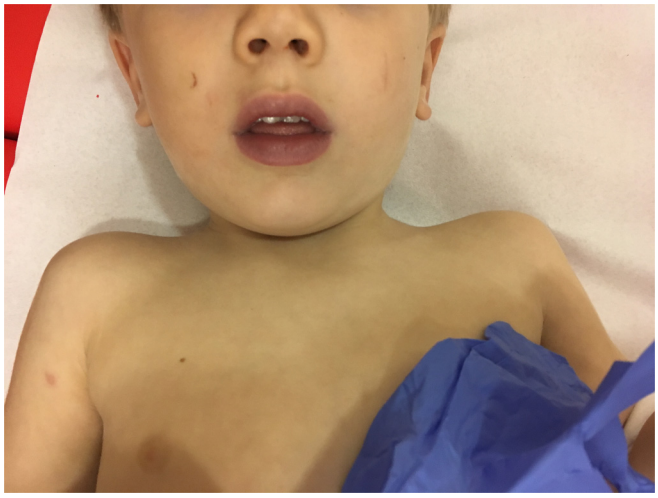

Fig. 1. Pseudo-cyanosis, brown coloration, more evident in patient's lips.

the peak were as follows: HbA: 72.9%; HbF: 2%; HbA2: 14.8% with a retention time of the HbA2 peak of 3.73 minutes (Fig. 2). Electrophoresis at alkaline pH (Sebia®) did reveal an abnormal variant of Hb in the HbE zone (Fig. 3). Given the suspicion of structural hemoglobinopathy, the study of alpha- and beta-globin genes was requested. The β-globin gene was amplified using the following two pairs of primers: β1D: 5′-CCT AAG CCA GTG CCA GAA G-3' (from nt −160 to + −142) and CD2: 5′-GAC CTC CCA CAT TCC CTT TT-3' (from nt +1659 to + 1643) (all nt positions are provided relative to the CAP site = nt1 from NCBI GenBank). PCR products were treated with the ABI PRISMTM BigDye Terminator V1.1 Cycle Sequencing Ready Reaction Kit (PE Applied BioSystems, Foster City, CA, USA) with β1D and CD2 primers for sequencing, following the manufacturer's instructions and the sequence was analyzed on an

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	2.0*	---	1.09	21675
Unknown	---	1.0	1.25	11150
P2	---	3.3	1.37	36645
P3	---	4.3	1.78	48383
Unknown	---	0.3	2.14	3458
Ao	---	72.9	2.58	818030
A2	14.8*	---	3.73	183345

Total Area: 1,122,685

F Concentration = 2.0* %
A2 Concentration = 14.8* %

*Values outside of expected ranges

Analysis comments:

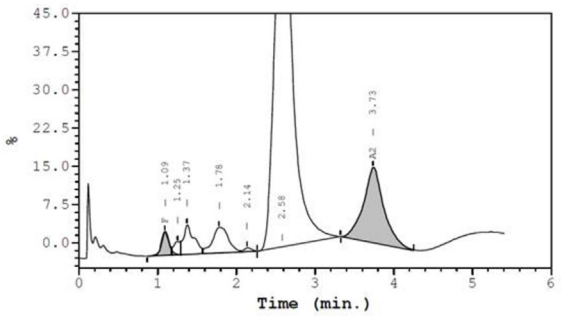

Fig. 2. High-performance liquid chromatography (VARIANT™). Retention time of the HbA2 peak of 3.73 minutes.

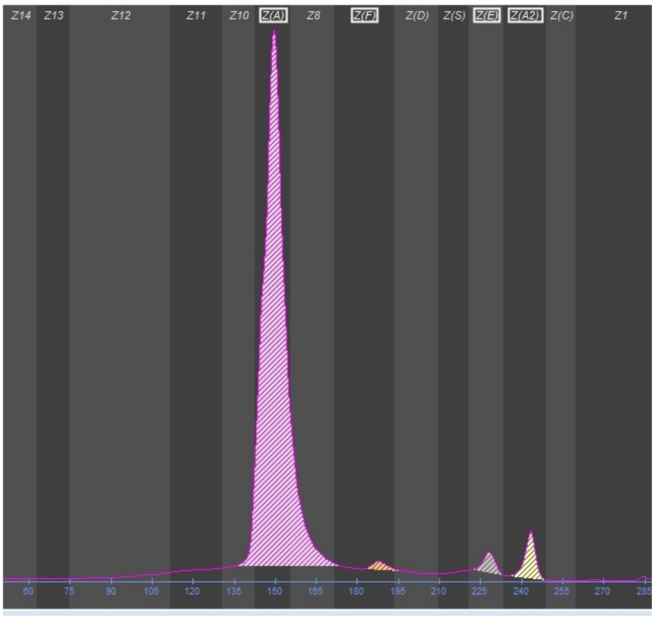

Fig. 3. Electrophoresis at alkaline pH (Sebia®) with an abnormal variant of Hb in the HbE zone.

ABI PRISM™ 310 Genetic Analyzer (PE Applied BioSystems) (Fig. 4). In the reference laboratory, where the hemoglobinopathies are studied, all the samples that are received are screened for alpha thalassemia by "multiplex PCR followed by reverse-hybridisation with a commercial Alpha-Globin StripAssay kit (ViennaLab Diagnostic GmbH, Vienna, Austria). The patient was finally diagnosed with a heterozygous hemoglobin M Saskatoon [Beta63 (E7) His>Tyr; HBB: c 190C> T] disease associated with alpha^{+} thalassemia 3.7 kb deletion in heterozygosity ($-\alpha^{3.7}/\alpha\alpha$). The parents' blood cell count was normal, had normal coloration and the study of the beta-globin gene in both was normal.

3. Discussion

Methemoglobinemia is a clinical syndrome characterized by increased in the percentage of Hb in which heme iron is oxidized to the ferric form (Fe^{3+}). It is caused by the presence of HbM or an increase in methemoglobin (MetHb).

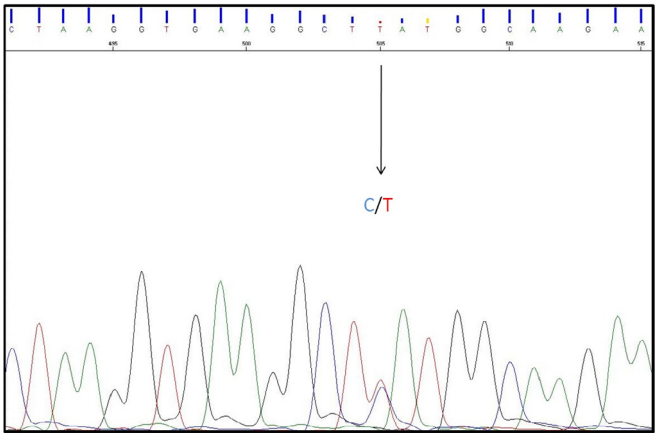

Fig. 4. Nucleotide sequence of part of the β-globin gene. Study of the mutation that results in HbM Saskatoon [Beta63 (E7) His>Tyr; HBB: c 190C> T].

MetHb is a reversible oxidation product of normal hemoglobin A ($2\alpha2\beta$): under normal conditions, up to 3% is produced daily before being reduced again in parallel by the action of nicotinamide adenine dinucleotide (NADH) cytochrome *b*5 reductase until a level of 1% is maintained [2]. The increase in MetHb can arise from a genetic defect in red blood cell metabolism or be acquired. The acquired form is the more common and appears following exposure to various iron oxidants (lidocaine, procaine, nitroglycerin, sodium nitroprusside, nitric oxide, metoclopramide), the congenital form of MetHb is recessively inherited owing to deficiency of the enzyme NADH cytochrome *b*5 reductase [1,2].

HbM comprises a group of structural variants of hemoglobin usually produced by substitution of proximal or distal histidine with tyrosine in the alpha, beta, or gamma hemoglobin chains, in close proximity to the heme pocket. Tyrosine is covalently bound to heme iron which stays in the oxidized ferric state and cannot be reduced by the enzymatic systems of red blood cells, as opposed to MetHb [1,2]. Eight variants of HbM arising from involvement of the α chain (HbM Boston, HbM Iwate, HbM Yantai), β chain (HbM Milwaukee, HbM Hyde Park, HbM Saskatoon), and γ chain (HbF M of Osaka and HbF M of Fort Ripley) have been described [3–5]. HbM has low affinity for oxygen, and the hemoglobin dissociation curve shifts to the right, thus causing an increase in oxygen supply to the tissues [6,7]. Consequently, the oxygen sensor induces a decrease in the production of erythropoietin, leading to mild anemia. In addition, since HbM Saskatoon is slightly unstable, mild hemolysis may be present with increased reticulocytosis, as in the case we report (Hb, 10.9 g/dL; total reticulocytes, 112 000/mm^3). However the MetHb has an increase affinity for the oxygen leading to a decrease in oxygen supply to the tissues; hence the onset of symptoms such as asthenia, headache, and lethargy, respiratory depression, seizures, and even death if MetHb is greater than 30% [1].

Clinically, the distinction between pseudo-cyanosis (brown coloration) and true cyanosis can be difficult. Skin and mucosal cyanosis is usually a manifestation of lung or heart disease that occurs when the blood concentration of deoxyhemoglobin is ≥ 5 g/dL. The administration of oxygen therapy increases the fraction of inspired oxygen (FiO_2) and therefore oxygen saturation. However, MetHb produced by the oxidation of heme iron causes a brown coloration. In HbM, pseudo-cyanosis occurs because erythrocytes contain an abnormal pigment produced by the combination of iron with a variant of globin [1,7]. In MetHb and in HbM, what leads to enzymatic or structural problems, administration of oxygen does not increase hemoglobin saturation. Incubation of blood with methylene blue reduces MetHb to normal HbA but does not reduce HbM (structural problem) [2,7]. The patient we report carried a beta variant (HbM Saskatoon). Pseudo-cyanosis did not present at birth because the HbA concentration is low in newborns and beta-chain synthesis reaches a significant concentration at 6 months of age, with the substitution of HbF ($2\alpha2\gamma$) for HbA ($2\alpha2\beta$) [1,8]. Children with alpha HbM variants are symptomatic at birth, and pseudo-cyanosis is present throughout life [9]. When the defect lies in the gamma chains, symptoms appear at birth and disappear at 2 months of age, when HbF is replaced by increasing amounts of HbA [4].

Diagnosis of methemoglobinemia is based on co-oximetry, which emits a beam of light with 4 wavelengths that differentiates the 4 main types of Hb: oxygenated Hb (oxyhemoglobin), deoxygenated Hb (deoxyhemoglobin), carboxyhemoglobin, and MetHb [10]. The transcutaneous oxygen saturation values measured by a pulse oximeter (SpO_2) are not reliable because the pulse oximeter devices only emit light at 2 wavelengths for oxyhemoglobin and deoxyhemoglobin. In our patient, the co-oximetry study showed a normal PaO_2 (105 mmHg) with MetHb of 8.5%, although, theoretically, the absorption spectrum of HbM differs from that of MetHb [8], so the finally distinction between MetHb and HbM is based on a detailed clinical, personal, and family history. With the suspicion of structural hemoglobinopathy with low affinity for oxygen, HPLC was requested because of its accessibility and identified an abnormal peak like HbA2, although electrophoresis and a genetic study of the hemoglobin chains were necessary to confirm the diagnosis.

HbM Saskatoon characterized by the beta-globin mutation [β63 His> Tyr (C-T)] was first described in 1948. As with other types of HbM, it is an autosomal dominant inherit disorder, but with a high rate of spontaneous mutations [8,11,12]. As in the case we report, neither of the parents had had pseudo-cyanosis and both had a normal blood cell count. The prognosis is excellent in heterozygous individuals; HbM Saskatoon is not associated with dyspnea or acropachy and does not affect life expectancy [1,2,7]. A case with increased hemolysis after treatment with an oxidant has been reported; therefore, it is recommended to avoid exposure to agents that induce MetHb [8,13,14]. There is also a report of a child in whom this mutation was associated with another mutation in the beta chain, namely, Hamilton Hb (β^{11}Va> Ile, HBB: c.34G> A)/HbM Saskatoon, and the clinical course of the resulting disease is also characterized by mild hemolytic anemia [13]. A case of HbM Saskatoon has been reported in a child with suspected major beta-thalassemia disease, although since no mutation was identified, genetic confirmation was not possible. The patient died after cardiac arrest in the context of severe anemia (6.2 g/dL) [14]. As no mutation has been described in homozygosity, genetic counseling remains incomplete.

4. Conclusions

HbM should be considered in the differential diagnosis of cyanosis or pseudo-cyanosis once cardiorespiratory conditions have been ruled out. Patient assessment is based on arterial blood gas analysis with cooximetry, since transcutaneous oxygen saturation is not reliable. After the finding of MetHb, potential toxic agents need to be ruled out, and, in their absence, we should suspect NADH-cytochrome b5 reductase enzyme deficiency or M hemoglobinopathy. To distinguish them, the family history and a hemoglobin study may be helpful.

We report the first case of HbM Saskatoon in heterozygosity in Spain.

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Conflict of interest

The other authors have no conflicts of interest to disclose.

Contributors' statements

Marina García-Morin: Dr. García-Morin suspected the patient's diagnosis, drafted part of the manuscript, approved the final manuscript as submitted.

Gema Manrique-Martín: Dr. Manrique-Martín suspected the patient's diagnosis, drafted part of the manuscript, and approved the final manuscript as submitted.

Paloma Ropero: Dr. Ropero performed the electrophoresis and

HPLC of hemoglobin as well as the genetic diagnosis of the patient, drafted part of the manuscript and approved the final manuscript as submitted.

Eduardo Bardón-Cancho: Dr. Bardón Cancho suspected the patient's diagnosis, drafted part of the manuscript, and approved the final manuscript as submitted.

Reyes Álvarez García-Rovés: Dr. Álvarez García-Rovés ruled out cardiac pathology in the patient, drafted part of the manuscript and approved the final manuscript as submitted.

Cristina Beléndez: Dr. Beléndez suspected the patient's diagnosis, critically reviewed the manuscript, and approved the final manuscript as submitted.

Elena Cela: Dr. Cela suspected the patient's diagnosis, critically reviewed the manuscript, and approved the final manuscript as submitted.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Informed consent for the picture was obtained from the patient's parents.

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