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Hemoglobin Denver, a cause of desaturated pulse oximetry. A pediatric case report

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ABSTRACT

Hemoglobinopathies are genetic disorders that affect the hemoglobin (Hb) molecule. Mutations in the alpha or beta chains altering the Hb tetramer may modify the molecule's oxygen-binding capacity. Hemoglobinopathies with low oxygen affinity may occur with cyanosis and an altered pulse oximetry reading, leading to unnecessary and sometimes invasive tests to rule out cardiovascular and respiratory conditions.

In the case report described here, we present an asymptomatic pediatric patient who consulted for desaturated pulse oximetry. Her initial laboratory tests showed normocytic, normochromic anemia. Venous blood gas samples showed an elevated p50. After using extensive diagnostic tools, a variant of Hb with low oxygen affinity was diagnosed: Hb Denver.

Keywords: *abnormal hemoglobins; hemoglobin Denver; transcutaneous blood gas monitoring.*

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INTRODUCTION

Hemoglobin A (HbA) is a tetramer of 2 alpha polypeptide chains (α) and 2 beta polypeptide chains (β), each bound to a heme prosthetic group. The main function is the regulation of oxygen (O_2), carbon dioxide, and nitric oxide transport. A fascinating characteristic of Hb, critical for its function, is its ability to bind oxygen cooperatively, giving the O_2 equilibrium curve a sigmoid shape. As O_2 binds to Hb, the affinity of O_2 to bind to other sites increases. The opposite occurs when deoxygenation takes place. O_2 saturation is approximately 98% in arterial blood, whereas it is 75% in venous blood. The value of the partial pressure of O_2 at which 50% of the O_2 binding sites in Hb are occupied is called p50.[1,2]

There are 2 stable states of the quaternary structure of Hb, with a difference in the orientation of the α1β1 and α2β2 dimers within the tetramer, defined as tense state (T) and relaxed state (R). The transition between the T state (low affinity state) and the R state (high affinity state) involves the cooperative binding of oxygen to Hb.

Hemoglobinopathies are the most common monogenic disorders resulting from pathogenic genetic variants of either the alpha-globin or beta-globin gene clusters, or both. Deletions or point mutations in these genes cause abnormalities in Hb synthesis or structure, resulting in alpha or beta thalassemia syndromes or structural variants of hemoglobin, respectively.[3] Hb variants are classified according to their effects on the molecule. Such classification includes unstable Hb and Hb with altered oxygen affinity.

A total of 151 hemoglobinopathies with altered oxygen affinity have been described. Although some variants affect the *HBA2* and *HBA1* genes, most affect mainly the *HBB* gene. Of these variants, 48 have low oxygen affinity.[3] If the reduction in O_2 affinity is low, these variants may be relatively asymptomatic; however, they may occasionally occur with cyanosis or hemolysis due to the instability of the Hb molecule.

Hemoglobinopathies with low O_2 affinity may occur with low saturation levels on pulse oximetry; however, this has no pathological significance because there is an increased delivery of O_2 to peripheral tissues. They usually present with normal partial pressure of oxygen in arterial blood (PaO_2), revealing absence of hypoxemia and tissue hypoxia. A potential Hb variant should always be taken into consideration in asymptomatic patients with desaturated pulse oximetry.[4]

Here we describe the case of a rare hemoglobinopathy as the differential diagnosis of desaturation.

CASE REPORT

A previously healthy 9-year-old girl consulted at our hospital for low saturation levels on pulse oximetry, measured by her mother, a nurse, while she was playing at home. She was completely asymptomatic. Her physical examination was normal and she did not show any symptoms of chronic hypoxemia. Her growth curve was normal. There was no family history of hypoxemia or low saturation levels on pulse oximetry.

On admission to our hospital, her saturation level measured with a pulse oximetry was 77%, with levels that rose to 97% with exogenous oxygen administration via nasal cannula, so the patient remained on exogenous oxygen. Her laboratory tests showed normocytic normochromic, non-regenerative anemia (Hb: 9.1 g/dL, mean corpuscular volume: 82.2 fl, mean corpuscular hemoglobin: 27.4 pg, reticulocytes: 1.3%). No characteristic morphological alterations were observed in the blood smear. A venous blood gas sample showed a p50 of 40.92 mmHg (normal value: 27 mmHg ± 2 mmHg). Carboxyhemoglobin and methemoglobin levels were normal. An arterial blood sample was obtained, with an oxygen saturation level of 90%. For reasons we do not understand at this time, we were unable to achieve a measurement of arterial oxygen levels, due to a consistent failure message in our blood gas sampling equipment (with multiple samples taken with and without exogenous oxygen administration).

The patient was assessed by the Departments of Pediatric Cardiology and Pulmonology. Arteriovenous shunts were ruled out by a computed tomography angiography of the chest, abdomen, and pelvis, and by a color Doppler echocardiogram. In turn, the latter ruled out congenital heart diseases.

The following tests were performed and no alterations were observed: capillary electrophoresis (Capillarys 2; Sebia, Lisses, France), electrophoresis in agar at pH 6.0, isopropyl alcohol and heat tests, and supravital staining to visualize Heinz bodies by standard methods.[5]

Laboratory tests and pulse oximetry were performed on both parents and no alterations were found, as shown in *Table 1*.

DNA was isolated from peripheral blood

leukocytes using the salting-out method,[6] and the *HBB* gene was amplified using primers previously described by Roldán et al.[7] The flanking exonic and intronic regions were directly sequenced with the Sanger method and an ABI PRISM 3500 capillary sequencer (AppliedBiosystems, Buenos Aires, Argentina). The reference sequence used for analysis was NG_000007.3 (http://www.ncbi.nlh. nih.gov/RefSeq/), which was performed using SeqScape v2.6 (AppliedBiosystems, Buenos Aires, Argentina).

As shown in *Figure 1*, the heterozygous variant NM_000518.5:c.125T>C (p.Phe42Ser) was found, which confirmed the diagnosis of hemoglobin Denver.

The variant was named according to the Human Genome Variation Society (HGVS) nomenclature for mutations.

Once the diagnosis was confirmed, oxygen administration was discontinued. Treatment with folic acid was started.

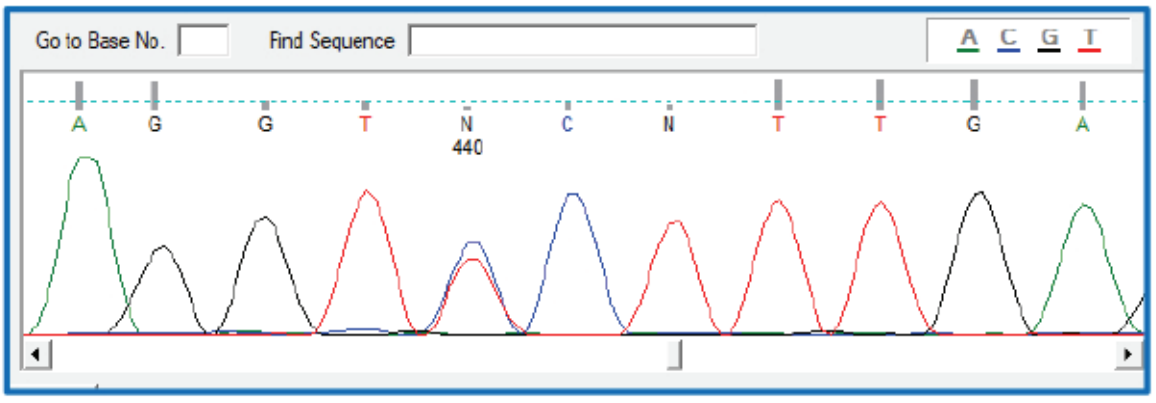
The patient currently performs artistic

Table 1. Laboratory parameters of the subject and her parents

Parameters	Subject (age: 9 years) (Reference values)	Father (age: 39 years) (Reference values)	Mother (age: 37 years) (Reference values)
RBC (10^6/mm^3)	3.6 (4.05–5.3)	5.15 (4.50–5.90)	3.82 (4.00–5.20)
Hb (g/dL)	9.7 (12.0–15.0)	15.6 (13.5–17.5)	12 (12.0–16.0)
MCV (fl)	80.6 (78.0–90.5)	87.8 (80–100)	91.6 (80–100)
RDW (%)	13.9 (11.4–13.4)	12.2 (11.4–13.6)	11.8 (11.4–14.4)
MCHC (g/dL)	33.4 (32.6–35.7)	34.5 (31.0–37.0)	34.3 (31.0–37.0)
MCH (pg)	26.9 (26.3–31.2)	30.3 (26.0–34.0)	31.4 (26.0–34.0)
Hematocrit (%)	29 (35.0–45.0)	45.2 (41.0–53.0)	35.0 (36.0–46.0)
Reticulocytes (%)	0.78 (0.5–2.5)	0.66 (0.5–2.5)	0.62 (0.5–2.5)
LDH (IU/L)	203 (less than 332)	175 (240–280)	200 (240–480)
TB (mg/dL)	0.44 (0.4–1.4)	0.56 (0.4–1.2)	0.46 (0.4–1.2)
DB (mg/dL)	0.18 (0.1–0.4)	0.22 (0.1–0.2)	0.24 (0.1–0.2)
Pulse oximetry (SpO_2) (%)	78 (98–100)	99 (98–100)	98 (98–100)
SaO_2 (%)	90 (98–100)	NP	NP
P50 (mmHg)	40.92 (25–29)	27.4 (25–29)	30.8 (25–29)
Isopropyl alcohol test	Negative	NP	NP
DNA sequence	NM_000518.5: c.125T>C (p.Phe42Ser)	ABSENCE OF NM_000518.5: c.125T>C (p.Phe42Ser) VARIANT	ABSENCE OF NM_000518.5: c.125T>C (p.Phe42Ser) VARIANT

RBC: red blood cells; Hb: hemoglobin; MCV: mean corpuscular volume; RDW: red cell distribution width; MCHC: mean corpuscular hemoglobin concentration; MCH: mean corpuscular hemoglobin; LDH: lactate dehydrogenase; TB: total bilirubin; DB: direct bilirubin; SpO_2: peripheral oxygen saturation; SaO_2: arterial oxygen saturation; P50: partial pressure of oxygen when the oxygen saturation is 50%; NP: not performed.

Figure 1. Sanger DNA sequencing of beta gene showing the NM_000518.5:c.125T>C (p.Phe42Ser) heterozygous variant



gymnastics and has no signs or symptoms of anemia. She continues with mild cyanosis on exertion. Her current complete blood count values include Hb: 11.1 g/dL, red blood cells: 4.09 × 10^6/mm^3, hematocrit: 35.3%, mean corpuscular volume: 86.3 fl, mean corpuscular hemoglobin: 27.1 pg/mL, mean corpuscular hemoglobin concentration: 31.4 g/dL, red cell distribution width (RDW): 13.6%, reticulocytes: 1.48%.

DISCUSSION

Only a few cases of Hb Denver have been reported in the bibliography; the first case was described in 1994.[8,9] Hb Denver is caused by a substitution in B41 (C7) Phe. The B41 (C7) position corresponds to a heme contact, and the substitution of hydrophobic phenylalanine for hydrophilic serine affects O_2 binding, causing instability of Hb and resulting in a substantial reduction in O_2 affinity.[8]

A reduced O_2 affinity of certain hemoglobin variants is usually manifested by the presence of cyanosis. Cyanosis caused by these variants is usually associated with normal arterial O_2 saturation and has no impact on patients' health. The relevance of an accurate and timely diagnosis lies in preventing unnecessary and potentially dangerous procedures to rule out other causes of cyanosis, including pulmonary and heart disease.

However, it has been described that, in the case of Hb Denver, there may be not only a reduction in O_2 affinity, but also a reduction in arterial O_2 saturation. The affinity for O_2 of the Hb molecule is so low that, at the PO_2 of arterial blood, the O_2 saturation is approximately the same as that observed in venous blood. However, the oxygen supply to the tissues is not affected.[8]

Hb Denver has not only been associated with the presence of cyanosis, as described above, but also with an unstable Hb molecule.

Hb instability may lead to different clinical manifestations, depending on its severity. Patients may be asymptomatic, have hemolytic anemia, or even a thalassemic phenotype.[10]

In our patient, it is worth noting the absence of cyanosis, the presence of anemia without clinical symptoms and without laboratory findings suggestive of hemolysis, and the absence of unstable Hb on laboratory tests.

With this case report, we aimed to describe the clinical manifestations that differ from those already described in the bibliography with such rare hemoglobinopathy.

We highlight the importance of a strong awareness when seeing a patient with a low pulse oximetry value who presents with normal initial cardiac and pulmonary tests, in order to be able to suspect and thus study hemoglobin variants and therefore avoid diagnostic tools that may be invasive and potentially harmful.

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