ABSTRACT. Cyanosis is a physical finding that can occur at any age but presents the greatest challenge when it occurs in the newborn. The cause is multiple, and it usually represents an ominous sign, especially when it occurs in association with neonatal sepsis, cyanotic congenital heart disease, and airway abnormalities. Cyanosis caused by abnormal forms of hemoglobin can also be life-threatening, and early recognition is mandatory to prevent unnecessary investigations and delay in management. Abnormal hemoglobin, such as hemoglobin M, is traditionally discovered by electrophoresis, so the newborn screen, which is mandatory in several states, is a useful tool for the diagnosis. Although acquired methemoglobinemia, caused by environmental oxidizing agents, is common, congenital deficiency of the innate reducing enzyme is so rare that only a few cases are documented in the medical literature around the world. We present a neonate with cyanosis as a result of congenital deficiency of the reduced nicotinamide adenine dinucleotide-cytochrome b5 reductase enzyme. This infant was found to be blue at a routine newborn follow-up visit. Sepsis, structural congenital heart disease, prenatal administration, and ingestion of oxidant dyes were excluded as a cause of the cyanosis by history and appropriate tests. Chocolate discoloration of arterial blood provided a clue to the diagnosis. A normal newborn screen and hemoglobin electrophoresis made the diagnosis of hemoglobin M unlikely as the cause of the methemoglobinemia (Hb A 59.4%, A, 1.8%, and F 38.8%). Red blood cell enzyme activity and DNA analysis revealed a homozygous form of the cytochrome b5 reductase enzyme deficiency. He responded very well to daily methylene blue and ascorbic acid administration, and he has normal growth and developmental parameters, although he has been established.6

CASE REPORT

A 24-day-old 3.4-kg infant was seen in the office for a routine postnatal follow-up appointment. He was born at full term from spontaneous vaginal delivery to a 26-year-old gravida 5 mother with full antenatal care and no perinatal problems. Apgar score was 8 and 9 at 1 and 5 minutes, respectively. He was discharged from the hospital on the second day after his first hepatitis B immunization. He had been well since discharge, taking approximately 4 oz of premixed formula every 4 hours, and his development had been appropriate. His mother did not recognize that his coloration was unusual and denied use of any medications. Parental consanguinity was denied.

Mild central cyanosis was noted on physical examination. Pulse oximetry was 87% in room air, so he was transferred to the emergency department of our regional children’s hospital. In the emergency department, the vital signs were T° 99°F, heart rate 182/min, respiratory rate 36/min, saturation on pulse oximetry 91%, with fraction of inspired oxygen 1.0 and a Dextrostix of 78 mg/dL. Blood pressure measurement could not be obtained, despite rapid infusion of 20 mL/kg crystalloid fluids. He continued to appear dusky and cyanotic and was electively intubated with a presumed diagnosis of septic shock. Appropriate cultures were obtained, broad-spectrum intravenous antibiotic coverage was started, and he was transferred to our multidisciplinary pediatric intensive care unit.
Chest radiograph revealed a normal heart shadow with clear lung fields. Echocardiography confirmed a normal cardiac anatomy with adequate function. On obtaining femoral arterial access, his blood had a dark chocolate appearance despite documentation of arterial pulsations and a transduced arterial wave form on the cardiorespiratory monitor. His initial hematocrit was 20%, probably iatrogenic as a result of technical difficulty in obtaining vascular access and multiple fluid boluses, so he was given a 20 mL/kg packed red blood cell (PRBC) transfusion, which improved his hematocrit to 30%.

Arterial blood gas analysis revealed a pH 7.42/Paco2 334/HCO3 24/base deficit −2 with a calculated hemoglobin saturation of 100% on fraction of inspired oxygen 1.0. Serum lactic acid was 1.2 mmol/L (n = 0.5–1.6). Serum methemoglobin level was 26.0% and carboxyhemoglobin was 0.2% by co-oximetry. There was no clinical or culture support for sepsis, and he was quickly weaned off the ventilator and extubated to room air, which he tolerated very well with oxygen saturations on pulse oximetry ranging between 87% and 94%.

He received a diagnostic dose of methylene blue, 2 mg/kg on the second day of admission. He tolerated the dose without side effects. Methemoglobin level 12 hours after the methylene blue was 7.9% dropping to 1.5% 24 hours after the medication.

Over the next 3 days, monitored in the PICU, fed only pre-mixed infant formula, his methemoglobin level increased to 28.6% with no change in his clinical status. Daily serum lactic acid levels remained <2.0 mmol/L.

He developed diarrhea, averaging 8 stools/d with a subsequent increase in the methemoglobin level to 44% and significant metabolic acidosis. The diarrhea resolved with a change to soy-based formula, and his acidosis resolved with fluid replacement.

At this point, a deficiency in reduced nicotinamide adenine dinucleotide (NADH)-cytochrome b5 reductase was suspected, and whole blood samples from the infant and his parents and 2 full siblings were obtained. The samples were analyzed for cytochrome b5 reductase (methemoglobin reductase B) enzyme activity. This assay was performed spectrophotometrically by measuring the oxidation of NADH at a wavelength of 340 nm. The test was performed by the Mayo Clinics laboratories. His initial enzyme activity level was 7.2 IU/g (n 10.1–19.4 IU/g). This level, although low according to the established range at the Mayo Clinic, was falsely elevated as a result of the PRBC transfusion. The enzyme activity level was repeated after 3 months, an adequate interval for destruction of most of the transfused PRBC. The repeat level was 4.2 IU/g. This is consistent with a homozygous deficiency of the cytochrome b5 reductase enzyme (Table 1).

The infant was started on daily oral methylene blue (1.5 mg/kg) and ascorbic acid (5 mg/kg). Methemoglobin level remained 4.8%, and he was discharged from the hospital with only mild central cyanosis.

**DISCUSSION**

Methemoglobin is produced from oxidation of ferrous iron (Fe2+) to ferric iron (Fe3+) within the heme moiety of hemoglobin. Methemoglobin, which normally constitutes <1% of the total hemoglobin, cannot carry oxygen. Furthermore, as a consequence of allosteric interactions within the molecule, there is an increased affinity for oxygen at the remaining binding sites, causing a left shift in the oxygen dissociation curve. Both of these phenomena contribute to a reduction in the delivery of oxygen to tissues and, if severe enough, hypoxemia and lactic acidosis.

Elevation of the methemoglobin content of erythrocytes arises as a consequence of either an acceleration of an oxidation reaction or a diminution of a reduction reaction, ie, a redox imbalance. In cases of acquired methemoglobinemia, the erythrocytes are presented with an enormous exogenous oxidant load that simply overwhelms the protective cellular reduction mechanisms. Conversely, in cases of congenital methemoglobinemia, cytochrome b5 reductase activity is diminished and there is a resultant decrease in the rate of methemoglobin reduction. The oxidant load under these circumstances is derived from endogenous sources.

In 1845, Francois, a French physician, described a patient with enduring congenital cyanosis in the absence of any obvious cardiac or pulmonary dysfunction. Although this was the first documented case of congenital methemoglobinemia in the professional literature, it was not until 1932 that Hitzenberger recognized idiopathic cyanosis to be a familial ailment. In the 1940s, Gibson argued and subsequently showed that there was a diminution in the ability of the erythrocytes to reduce methemoglobin in such individuals.

In 1959, Scott and Griffith identified the enzyme responsible for reducing methemoglobin in normal erythrocytes. They called this NADH-requiring enzyme diaphorase. Now generally referred to as NADH-cytochrome b5 reductase, a functional deficiency in this enzyme is universally recognized as the underlying cause of congenital methemoglobinemia. In 1986, Jaffe proposed a clinical-biochemical classification scheme based on important differences in the pathophysiology of the disorder. Accordingly, he asserted that hereditary enzymopoenic methemoglobinemia could be stratified into 4 distinct classes or types. Type 1, the most common and least debilitating, involves a deficiency in cytochrome b5 reductase limited to erythrocytes. Type 2 congenital methemoglobinemia is more pervasive and is associated with a generalized systemic deficiency affecting a multitude of tissues, particularly the central nervous system. After additional study with a more sensitive assay, type 3 hereditary enzymopoenic methemoglobinemia was shown by Nagai et al to be virtually identical to type 1. As such, an independent type 3 classification was proved to be superfluous and is not currently used. Type 4 is unique in that it does not actually involve a deficiency in the cytochrome b5 reductase itself. This class of the disease, which has been reported only in a single case, is manifested by an attenuated concentration of cytochrome b5.

Type 1 presents with little more than visible cyanosis. In the words of Jaffe and Hultquist, “These patients are really more blue than sick.” Although the slate gray, bluish appearance of these infants may be alarming to physicians who are unaware of the underlying pathophysiology, the methemoglobinemia is usually well tolerated. These individuals generally do not become symptomatic until their methemoglobin levels exceed 25% of the total hemoglobin, and the most commonly reported symptoms are benign, including headache, fatigue, and exertional dyspnea.

**TABLE 1. NADH-Cytochrome b5 Reductase Activity**

<table>
<thead>
<tr>
<th></th>
<th>Father</th>
<th>Mother</th>
<th>Sibling A</th>
<th>Sibling B</th>
<th>Proband</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic Activity (IU/g)†</td>
<td>9.2</td>
<td>7.7</td>
<td>8.4</td>
<td>7.5</td>
<td>7.2</td>
</tr>
</tbody>
</table>

* Normal Mayo Clinics enzymatic activity range 10.1 to 19.4 IU/g.
† Repeat level 3 months after transfusion.

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Type 2 congenital methemoglobinemia does not run such a benign course. It constitutes approximately 10% of all cases and usually causes death within the first few years of life. The severity of disease is a direct consequence of the global deficiency in NADH-cytochrome b5 reductase activity that characterizes this class of the disorder. The distinguishing feature of type 2 and the sine qua non is an unremitting, progressive neurologic deterioration. First described in a paper published in the British Medical Journal, this fulminant disease is associated with mental retardation, microcephaly, opisthotonus, athetoid movements, and generalized hypertonia.

Individuals with congenital methemoglobinemia will typically present with cyanosis in the neonatal period. In managing a cyanotic patient, physicians will often obtain an arterial blood gas analysis, in addition to monitoring pulse oximetry. Unfortunately, the patient with methemoglobinemia will often have normal values for both. In interpreting arterial blood gas data, the clinician must remember that the PaO2 refers to the amount of dissolved oxygen in the blood and in no way reflects hemoglobin saturation and thus arterial oxygen content. Patients with life-threatening methemoglobinemia may have a normal PaO2 and a falsely elevated pulse oximetry reading.

Unlike a pulse oximeter, which measures light absorbance at 2 wavelengths (660 nm and 940 nm, corresponding to the absorption of oxyhemoglobin and deoxyhemoglobin, respectively), a co-oximeter measures light absorbance at 4 different wavelengths. These wavelengths correspond to the absorption characteristics of deoxyhemoglobin, oxyhemoglobin, carboxyhemoglobin, and methemoglobin. As a consequence, co-oximetry can distinguish between these 4 configurations while providing a more accurate measurement of oxygen saturation. Therefore, in patients who present with cyanosis of uncertain cause, co-oximetry measurements are a valuable diagnostic tool.

Hemoglobin electrophoresis is also a very helpful adjunct in differentiating the different causes of congenital cyanosis. It will identify hemoglobin M, a hemoglobin variant that causes cyanosis as a result of structural changes in the α or β chains that stabilize the hemoglobin in the ferric state. These structural changes are attributable to amino acid substitutions at positions close to the heme groups in the hemoglobin molecule. Cyanosis is noticed at birth or within 4 to 6 months thereafter.

Once the diagnosis of methemoglobinemia has been made, there are various assays available to quantify NADH-cytochrome b5 reductase activity. Adult levels of enzyme function are attained by 2 to 3 months of age, and in the neonate, methemoglobin reductase levels are normally 60% of the normal adult value. When congenital methemoglobinemia is suspected, enzyme activity in all immediate family members should be evaluated. As a result of autosomal recessive transmission, by definition our patient must have both alleles, and, accordingly, each parent contributes 1 allele. In heterozygous deficiency, methemoglobin reductase activity is low, as seen in both parents of our patient. Heterozygotes (both parents and the 2 siblings) will have a lower threshold for acquired methemoglobinemia in response to exogenous oxidative stress. However, their level of enzyme activity is not low enough to produce clinical disease under normal circumstances. All other members of our patient’s family had methemoglobin levels below 2%.

In the treatment of hereditary enzymopenic methemoglobinemia, many variables have to be taken into consideration. Often, patients will remain completely asymptomatic. However, methemoglobinemia causes a leftward shift of the oxygen-hemoglobin dissociation curve. Furthermore, in the neonatal period, there is a persistence of fetal hemoglobin and a more pronounced difficulty of oxygen dissociation at the cellular level. These factors, combined with the deleterious effects of reduced arterial oxygen content in the neonatal period, make it reasonable to attempt to keep the methemoglobin level under 10% during this period.

Methylene blue is the treatment of choice for severe methemoglobinemia. In the presence of nicotinamide adenine dinucleotide phosphate (NADPH), methylene blue is converted to leucobethylene blue, which results in nonenzymatic reduction of methemoglobin. Ascorbic acid directly reduces methemoglobin, but the rate of the reaction is too slow for it to be effective when used alone. Finally, if the combination of ascorbic acid and methylene blue fails to reduce the methemoglobin level, then hyperbaric oxygen and exchange transfusions are alternative therapy.

Our patient demonstrated all of the classical features of congenital methemoglobinemia on presentation. He was treated in the emergency department as a child in septic shock as a result of the usual presentation of a rare disease. His initial level of the cytochrome b5 reductase enzyme level was skewed as a result of the PRBC transfusion. A repeat level 3 months after the transfusion revealed his actual enzyme level of 4.2. It is impossible at this point to determine whether he will be classified as having type 1 or 2. This has significant prognostic implications, and full genetic analysis of the family is in progress. In summary, congenital methemoglobinemia is a very rare but treatable cause of neonatal cyanosis that should be considered in the differential diagnosis of cyanosis and septic shock in the neonatal period.

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Congenital Methemoglobinemia: A Rare Cause of Cyanosis in the Newborn—A Case Report
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