**CASE DESCRIPTION**

A 33-year-old woman of Indian origin presented for prenatal care at term. An ultrasound report from earlier in her pregnancy indicated complete placenta previa and placenta increta, which were confirmed by repeat ultrasound. She was admitted to the hospital for monitoring and delivery planning.

On admission, brown vaginal spotting was noted on the patient’s pad. Her nail beds and fingers were cyanotic, and a finger prick blood sample was rusty brown in appearance. Oxygen saturation by pulse oximetry on room air was 45% despite her appearing clinically well. She was otherwise asymptomatic. The patient’s husband remarked that her family has “brown blood, not red,” noting that the patient’s father, paternal grandfather, and sister also have brown blood but no clinical problems. Her 3-year-old daughter had a similar cyanotic appearance but was otherwise asymptomatic.

Repeat oxygen saturation measurements by pulse oximetry consistently gave results between 40%–60%, and arterial blood \(P_{O_2}\) was 95 mmHg. Methemoglobin measurement was attempted, but the cooximeter indicated “Oximetry measuring error.” This was also confirmed by repeated measurements.

Complete blood count results were as follows: hemoglobin, 12.5 g/dL (125 g/L; reference interval, 120–160 g/L); red blood cells (RBC), \(6 \times 10^{12}/L\) (reference interval, 3.80–5.20 \(\times 10^{12}/L\)); hematocrit (HCT), 38% (0.38; reference interval, 36–46); mean corpuscular volume (MCV), 90 fl (reference interval, 80–100 fl); mean corpuscular hemoglobin (MCH), 30 pg (reference interval, 26–35 pg); mean corpuscular hemoglobin concentration (MCHC), 33.3 g/dL (333 g/L; reference interval, 310–360 g/L); red cell width distribution (RDW), 13.2% (reference interval, <15.6%); platelets (PLT), \(224 \times 10^9/L\) (reference interval, 140–450 \(\times 10^9/L\)); white blood cells (WBC) 9.6 \(\times 10^9/L\) (reference interval, 4.0–11.0 \(\times 10^9/L\)).

A peripheral blood film showed mild anemia with microcytes, no significant polychromasia or poikilocytosis, and neutrophils with prominent granularity. There was no increase in reticulocyte count, and glucose-6-phosphate dehydrogenase activity was normal.

The newborn child was also found to be cyanotic at birth, with brown blood similar to the mother’s. Attempts to measure methemoglobin were once again unsuccessful, and oxygen saturation results by pulse oximetry were consistently very low despite normal vital signs. Complete blood count results for the infant were as follows: hemoglobin, 17.2 g/dL (172 g/L; reference interval, 135–195 g/L); RBC, \(4.66 \times 10^{12}/L\) (reference interval, 3.90–5.50 \(\times 10^{12}/L\)); HCT, 51% (0.51; reference interval, 0.42–0.60); MCV, 110 fl (reference interval, 88–118 fl); MCHC, 37 pg (reference interval, 26–35 pg); MCHC, 33.5 g/dL (335 g/L; reference interval, 330–360 g/L); RDW, 16.0% (reference interval, <15.6%); PLT, 176 \(\times 10^9/L\) (reference interval, 140–450 \(\times 10^9/L\)); WBC, 12.5 \(\times 10^9/L\) (reference interval, 9.0–30.0 \(\times 10^9/L\)).

**QUESTIONS TO CONSIDER**

1. What are causes of cyanosis and brown blood?
2. What additional laboratory tests could be useful in this case?
3. What could cause both the pulse oximeter and cooximeter to give unusual results?

**DISCUSSION**

Cyanosis typically results from increased amounts of deoxyhemoglobin in the bloodstream. This may be due to hypoxemia, methemoglobinemia, or sulfhemoglobinemia (1). Methemalbuminemia and deposition of pigmented substances in skin may also cause cyanosis. Of these potential causes, methemoglobin and methemalbumin have absorption properties that give the blood a brown or chocolate brown color when present in sufficient amounts (1). However, methemalbumin forms as a result of excessive red blood cell destruction, which is not consistent with the patient’s hematological studies.

### References

1. DynaLIFEdx, Alberta, Canada.
2. Department of Laboratory Medicine and Pathology, University of Alberta.
3. Royal Alexandra Hospital, University of Alberta.
5. Address correspondence to this author at: DynaLIFEdx, #200, 10150-102 St., Edmonton, Alberta, Canada. Fax 780-454-2845; e-mail mathew.estey@dynalifedx.com.

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*Nonstandard abbreviations: RBC, red blood cells; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell width distribution; PLT, platelets; WBC, white blood cells; M, met; Hb M, hemoglobin M.*
Methemoglobin is formed through the oxidation of the heme iron from Fe$^{2+}$ to Fe$^{3+}$. This form of hemoglobin cannot bind oxygen, and it increases the oxygen affinity of the other heme groups within the hemoglobin tetramer. Consequently, methemoglobin causes cyanosis and impairs oxygen delivery to tissues. The signs and symptoms of methemoglobinemia vary depending on the fraction of methemoglobin. Methemoglobin values of 10%–20% cause clinically obvious cyanosis and brown blood; however, the patient may be otherwise asymptomatic. Methemoglobin above 30% may cause dyspnea, nausea, and tachycardia, whereas lethargy, syncope, loss of consciousness, and coma may occur when methemoglobin reaches 55% (2). Methemoglobin above 70% is incompatible with life. Under normal circumstances, the vast majority of hemoglobin (approximately 98%) is held in the reduced state. This is achieved predominantly through reduction of methemoglobin in an NADH-dependent manner by an enzyme called methemoglobin reductase (also known as NADH-cytochrome b5 reductase).

Methemoglobinemia may be due to either acquired or congenital causes. In most instances, consumption of an exogenous oxidizing agent causes oxidative stress, which overwhelms the body’s capacity to hold hemoglobin in the reduced state. Both chemicals (such as aniline dyes, pesticides, and fava beans) and medications (including dapsone, nitrous oxide, and quinine-based antimalaria drugs) can cause acquired methemoglobinemia (3). Congenital methemoglobinemia may be due to defects in methemoglobin reductase. Alternatively, certain hemoglobin mutations shift the equilibrium toward the oxidized state, thereby also causing congenital methemoglobinemia. These hemoglobin variants are referred to as M (met) hemoglobins (Hb M).

Patients with congenital methemoglobinemia typically have methemoglobin in the range of 15%–20%, whereas much higher amounts may occur in acquired methemoglobinemia.

Given the positive family history of brown blood, a congenital cause of methemoglobinemia was suspected. The patient’s methemoglobin reductase activity was found to be within the reference interval, ruling out methemoglobin reductase deficiency. Consequently, the presence of a hemoglobin variant was investigated.

![Fig. 1. Hemoglobinopathy investigation reveals the presence of an α chain variant.](image-url)
Hemoglobin analysis by HPLC revealed an abnormal peak with a retention time of 4.72 min, comprising 17.3% of the total hemoglobin (Fig. 1A). A small shoulder peak with a slightly longer retention time than the parent peak, which is characteristic of α-chain hemoglobin variants, was noted. Interestingly, the chromatography profile did not resemble that of any Hb M found in 2 independent databases of hemoglobin variants [Bio-Rad library of variants and Variant Haemoglobins: A Guide to Identification (4)]. The best putative match according to both of these databases was Hb Q-India (Table 1). HPLC analysis of the newborn’s blood also suggested the presence of an α chain hemoglobin variant (data not shown). The presence of cyanosis and brown blood in the neonate further supports an α chain variant, since a β chain mutation would present several months later due to low β chain expression at birth.

As recommended by the British Journal of Hematology guidelines for screening and diagnosing hemoglobin variants (5), an alternate method for hemoglobin identification based on a different analytical principle was performed. Electrophoresis at alkaline pH revealed bands in the A and S positions (Fig. 1B), which is consistent with Hb Q-India trait (4). However, electrophoresis at acid pH also revealed bands in the A and S positions (Fig. 1C), whereas a single band in the A position would be expected for the Hb Q-India trait (4). An Indian colleague was consulted and felt that the available data (Table 1) were most consistent with the presence of Hb Q-India. She noted that “brown blood disease” is frequently found in certain parts of India and is associated with the presence of Hb Q-India.

Since there is no literature demonstrating a causative link between Hb Q-India and methemoglobinemia, gene sequencing was performed to definitively identify the hemoglobin variant. The results indicated heterozygosity for Hb M-Boston (HBA2 c.175C>T), a rare α chain variant first described in the late 1950s and early 1960s (6, 7). Whereas the chromatographic properties of Hb M-Boston on the BioRad Variant Classic have been described previously (8), characterization of its mobility relative to other hemoglobin variants using modern electrophoresis systems is lacking.

The single His58Tyr mutation in Hb M-Boston results in a conformational change in the α subunit that explains many of the clinical findings observed in this case. First, the conformational change stabilizes the hemoglobin tetramer in the deoxy state (9), thus reducing the oxygen affinity and causing cyanosis. Second, the conformational change renders Hb M-Boston resistant to reduction by methemoglobin reductase (10). This causes methemoglobinemia, which both contributes to the cyanosis and accounts for the presence of brown blood. Finally, the conformational change also alters the absorption spectrum of Hb M-Boston relative to both Hb A and methemoglobin A (11). Because measurements of oxygen saturation and methemoglobin are based on the absorbance of Hb A at characteristic wavelengths, the presence of a hemoglobin variant with different absorption properties will interfere with these measurements (11).

Thus, the presence of Hb M-Boston also explains the severely underestimated oxygen saturation results and the inability to measure methemoglobin. Although this is also the case for other M hemoglobins, it is not an issue in acquired methemoglobinemia or methemoglobinemia by pulse oximetry and cooximetry may be unreliable or unobtainable in the presence of Hb M.

| Table 1. Chromatographic and electrophoretic properties of the patient’s hemoglobin variant and those expected for Hb Q-India. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Patient         | Hb Q-India*     | Patient         | Hb Q-India*     | Patient         | Hb Q-India*     | Patient         | Hb Q-India*     | Patient         | Hb Q-India*     | Patient         | Hb Q-India*     | Patient         | Hb Q-India*     | Patient         | Hb Q-India*     | Patient         | Hb Q-India*     | Patient         | Hb Q-India*     | Patient         | Hb Q-India*     | Patient         | Hb Q-India*     | Patient         | Hb Q-India*     | Patient         | Hb Q-India*     |
| HPLC            |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
| Variant, %      | 17.3            | 17.2            | Variant, %      | 17.3            | 17.2            | Variant, %      | 17.3            | 17.2            | Variant, %      | 17.3            | 17.2            | Variant, %      | 17.3            | 17.2            | Variant, %      | 17.3            | 17.2            | Variant, %      | 17.3            | 17.2            | Variant, %      | 17.3            | 17.2            |
| Variant retention time, min | 4.72            | 4.78            | Variant retention time, min | 4.72            | 4.78            | Variant retention time, min | 4.72            | 4.78            | Variant retention time, min | 4.72            | 4.78            | Variant retention time, min | 4.72            | 4.78            | Variant retention time, min | 4.72            | 4.78            | Variant retention time, min | 4.72            | 4.78            | Variant retention time, min | 4.72            | 4.78            | Variant retention time, min | 4.72            | 4.78            |
| Electrophoresis |                 |                 | Electrophoresis |                 |                 | Electrophoresis |                 |                 | Electrophoresis |                 |                 | Electrophoresis |                 |                 | Electrophoresis |                 |                 | Electrophoresis |                 |                 | Electrophoresis |                 |                 | Electrophoresis |                 |                 | Electrophoresis |                 |                 |
| Acid pH         | S position      | A position      | Acid pH         | S position      | A position      | Acid pH         | S position      | A position      | Acid pH         | S position      | A position      | Acid pH         | S position      | A position      | Acid pH         | S position      | A position      | Acid pH         | S position      | A position      | Acid pH         | S position      | A position      | Acid pH         | S position      | A position      | Acid pH         | S position      | A position      |

* Bain et al. (4).

**POINTS TO REMEMBER**

- Patients with methemoglobinemia may present with cyanosis and brown blood.
- Methemoglobinemia may be acquired (drug or chemically induced) or inherited (methemoglobin reductase deficiency or Hb M).
- The cause of methemoglobinemia may be assessed by measuring methemoglobin and methemoglobin reductase activity, and by performing a hemoglobinopathy investigation (acquiring a family, medication, and chemical exposure history are also critical).
- HPLC and gel electrophoresis (at both acid and alkaline pH) play an important role in identifying hemoglobin variants. However, different variants may have similar profiles using some or all of these techniques. Gene sequencing provides definitive identification, and is especially useful in identifying rare variants.
- Oxygen saturation measurement by pulse oximetry and methemoglobin measurement by cooximetry may be unreliable or unobtainable in the presence of Hb M.
globin reductase deficiency (since methemoglobin A is produced in these instances).

This case confirms that definitive identification of a hemoglobin variant is not always possible using HPLC and electrophoresis, even when both techniques are used in parallel. Indeed, with the use of these techniques alone, Hb M-Boston could easily be mistaken for Hb Q-India. To our knowledge, this is the first description of Hb M-Boston in a patient of Indian ethnicity. Given the lack of evidence demonstrating a causative link between Hb Q-India and methemoglobinemia, it is tempting to speculate that Hb M-Boston may actually be more prevalent in Indian populations, yet is being misidentified as Hb Q-India.

This case highlights 3 very valuable points. These are the importance of correlating laboratory findings with the clinical presentation, the value of multiple methods for the identification of hemoglobin variants, and the appropriate use of molecular testing.

With the patient’s clinical presentation, family history, abnormal pulse oximetry readings, and the presence of a hemoglobin variant on electrophoresis, the authors are commended for strongly suspecting an M hemoglobin. However, they were led astray by the HPLC results, making an identification of the variant as Hb Q-India even though the acid electrophoresis migration was inconsistent and Hb Q-India is not associated with clinical symptoms.

Fortunately, the authors sent the sample for molecular confirmation in which the hemoglobin variant was identified as Hb M-Boston, a result fully consistent with the clinical presentation. The authors indicate that the HPLC retention time of Hb M-Boston is not known; however, a very large series of the retention times of hemoglobin variants was published in 2012, which included 6 cases of Hb M-Boston, with molecular confirmation (1). In that study the retention time of Hb M-Boston (mean retention time, 4.80

**References**

min; range 4.74–4.85 min) was consistent with the retention time in this case. The slight difference in retention time may be because the study described in (1) compiled retention times on the Bio-Rad Variant Classic, and not the Bio-Rad Variant II, as reported here. In addition, Hb M-Boston does migrate close to the Hb S position on acid electrophoresis.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

References

Commentary

Neil S. Harris,* Stacy G. Beal, and William E. Winter

In the atmosphere at 20 °C, oxygen has a concentration of approximately 8.8 mmol/L, which matches the concentration of hemoglobin monomers in the blood. Therefore, when fully saturated, hemoglobin allows blood to achieve “atmospheric” oxygen concentrations.

Hemoglobin is remarkable for its exquisitely fine-tuned binding site that allows binding of oxygen without immediately oxidizing the ferrous iron (Fe$^{2+}$) to the ferric form (Fe$^{3+}$), while at the same time diminishing the very high binding affinity of carbon monoxide (CO), which is continuously generated by heme degradation. The oxygen binding site contains the heme porphyrin ring, which is bound to Fe$^{2+}$, forming a pentacoordinate complex: 4 covalent bonds from the porphyrin and 1 from the “proximal” (F8) histidine. Binding of oxygen is to the sixth coordination position of heme; this binding places the oxygen at an angle relative to the plane of the heme and allows the oxygen to hydrogen bond to the so-called “distal” (E7) histidine.

The hemoglobin M variants arise mainly as a result of the substitution of the proximal or distal histidines by tyrosine. These include Hb M-Boston and Hb M-Saskatoon (α and β chain distal histidine substitutions, respectively) and in Hb M-Iwate and Hb M-Hyde Park (α and β chain proximal histidine substitutions). Furthermore, there is an important role for a valine residue on the β chain, demonstrated in Hb M-Milwaukee, in which valine-E11 is replaced by glutamic acid. Hemoglobin M is not only resistant to the normal reduction mechanisms; in hemoglobins Boston and Iwate (both α substitutions), the oxygen affinity of the normal β subunit is very low.

Hemoglobin M variants present a challenge to the clinical chemist because they likely will be misread by oximeters, as described in this case. Our interpretations are only as good as our understanding of the biology of disease.

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Affiliations
Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, Gainesville FL 32610-0275.

* Address correspondence to this author at: University of Florida, P.O. Box 100275, Gainesville, FL 32610-0275. Fax 352-265-0447; e-mail harris@pathology.ufl.edu.

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