Case Series

Methylene blue for hemolytic crisis in patients with met-hemoglobinemia secondary to hemoglobin volga: A case series

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Abstract

There is a rare subset of patients with a genetically abnormal hemoglobin structure initially discovered in the Volga region of Europe known as Volga anemia. Key features of this condition include compromised delivery of oxygen to peripheral tissues and altered red blood cells that have a higher likelihood of being broken down or hemolyzed, which can lead to significant hemolytic anemia. Methylene blue is a dye that acts as a reducing agent of oxygen and is commonly used in toxic states that lead to methemoglobin build-up. This paper explores the pathophysiology of this genetic condition and documents three cases across two patients—a father and son—when methylene blue was used during an anemic crisis.

Keywords: Anemic crisis, hemolytic crisis, methylene blue, Volga anemia

Introduction

There is a rare subset of patients with a genetically abnormal hemoglobin structure initially discovered in the Volga region of Europe known as Volga anemia. We explore the pathophysiology of this condition and document three cases across two patients—a father and son—when methylene blue was used during an anemic crisis. While it is commonly used in toxic states that lead to methemoglobin build-up, there are no known cases of methylene blue being used to treat anemic crisis from Volga anemia and we wanted to raise awareness among primary care providers to improve time to diagnosis and intervention for future patients.

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Background

Methylene blue is a dye that acts as a reducing agent of oxygen; that is, it adds electrons to the oxygen molecule. It is approved for treating methemoglobinemia with the following dosing instructions: "Intravenous (IV): 1–2 mg/kg over 5–30 minutes (maximum single dose: 100 mg, using actual body weight); may repeat dose 1 hour later if methemoglobin level remains above 30% or symptoms persist."[1] It is often used as an antidote for toxin exposures that lead to methemoglobin build-up in the blood.[2]

Methemoglobin is an oxidized form of hemoglobin; that is, an electron was removed. When the iron molecule of blood is in the oxidized ferric (Fe^{3+}) state rather than the natural ferrous (Fe^{2+}) state, it causes oxygen to bind more tightly to blood cells, making it nearly impossible for oxygen to disband when needed by body tissues and leading to "functional anemia."[3] Some common agents known to cause this abnormal hemoglobin formation

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include nitrites and nitrates, bromates and chlorates, aniline derivatives, antimalarial agents, dapsone, propanil (an herbicide), sulfonamides, and local anesthetics.[4]

Signs of methemoglobin toxicity include lethargy, headache, dizziness, and nausea. With greater concentrations, these progress to dyspnea, confusion, seizures, and coma. Symptom severity typically correlates with measured methemoglobin levels, and even low levels may cause discoloration ("chocolate cyanosis") of the nails, lips, and ears.[4] A common phenomenon associated with methemoglobinemia is hemolysis due to the burden of oxidative stress on the protein portion of hemoglobin. This results in the denaturation of hemoglobin and the attachment of the damaged protein to the internal cell membrane. Cells containing denatured hemoglobin are either removed from circulation by the spleen or may be subject to premature lysis.[5]

In addition to toxin exposure, there is a rare subset of patients genetically predisposed to developing methemoglobinemia. One such condition is known as Volga anemia due to its discovery in the Volga region of Europe. It is caused by a genetic mutation of GCC → GAC at codon 27 (Beta27(B9)) causing alanine (Ala) to be substituted with aspartic acid (Asp).[6] This causes the non-polar, hydrophobic alanine to be replaced with aspartic acid, which can be hydrophilic in an acidic p^H.[7] This substitution is the catalyst that allows for the conversion of ferrous (Fe^{2+}) hemoglobin to ferric (Fe^{3+}) hemoglobin. This change causes the hallmark pathology of Volga anemia: An accumulation of hemoglobin in the methemoglobin configuration during times of physical stress on the body along with the same functional anemia, hemolysis, and clinical symptoms typically seen with toxic exposure. To our knowledge, methylene blue as a reducing agent for patients with Volga anemia has not been studied. Still, there is documentation of patients with hemoglobinopathies causing congenital methemoglobinemia that is unresponsive to methylene blue therapy.[5]

Case Presentations

Below we present three cases involving two patients who provided informed consent to share their cases—a father (Patient A) and his son (Patient B)—in which methylene blue treated Volga anemia crisis.

Case 1, Patient A - Father

Patient A is a 46-year-old African American male with a history of Volga anemia (diagnosed 2013), ulcerative colitis (diagnosed 2005), pulmonary embolism, pneumonia, osteoporosis, thoracic stress fracture, appendectomy, splenectomy (age 10 – unknown reason), and cholecystectomy (1999). He has a family history of untyped anemia in both parents who died from complications of anemia and renal disease before age 55. He has two sons and a daughter. Both sons have been diagnosed with Volga anemia but the daughter does not carry the mutation. Testing of Patient A in 2013 was negative for hemoglobin C or S, pyruvate kinase deficiency, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and osmotic fragility.

Before his diagnosis, Patient A presented to the hospital in 1999 for abdominal pain, fatigue, diffuse joint pain, and jaundice. With the cause of symptoms unknown, he underwent exploratory laparotomy and cholecystectomy. Soon after, he developed *E. coli* sepsis and was admitted to the Intensive Care Unit (ICU) and intubated. Lab values were notable for 11% methemoglobinemia (normal is <2%) and a high bilirubin. A blood smear showed oversized platelets, anisocytosis, and smudge cells. His hemoglobin averaged between 10 and 11 g/dL (normal is 13.4–17.5 g/dL), and he had thrombocytosis along with an elevated reticulocyte count and LDH levels. His haptoglobin was low, usually below 6 (normal is 32–197 mg/dL). His labs showed evidence of chronic hemolysis.

Initially, symptoms were attributed to likely toxin exposure due to the elevated methemoglobin. He received IV methylene blue (dosing information unavailable). The patient recovered after infusion, was extubated within 24 hours, and discharged soon after. Repeat methemoglobin level was not performed. It would not be until 2013 that his son's pediatric oncologist would order a hemoglobin analysis that led to the diagnosis of Volga anemia.

Case 2, Patient A - Father

In August 2021, Patient A was admitted to the hospital for the anemic crisis. His symptoms included lethargy/fatigue and body aches. His medications included apixaban for a COVID-19-provoked DVT, ciprofloxacin, and metronidazole for a recent ulcerative colitis-related infection, vitamin C, folate, polysaccharide iron complex, and vitamin E. He was not critically ill or in need of ICU services. His oxygen saturation (SPO_2) via pulse oximetry was 87%. His bloodwork at admission [see Table 1], then received a 68 mg infusion of methylene blue in 50 mL D5W, administered over 30 minutes (dosed at 1 mg/kg) starting on 8/4/2021 at 22:58. His lab values from the hospitalization and weeks following are also shown in Table 1. Patient A was discharged on 8/5/21 with lethargy and body aches resolved and SPO_2 via pulse oximetry at 91%. Soon after he revealed he had been taking an over-the-counter nitric oxide supplement before and for three weeks following hospitalization and methylene blue infusion.

Case 3, Patient B - Son

Patient B is a 20-year-old African American male with only Volga anemia in his medical history. In 2020, he was hospitalized five times between January and November for the anemic crisis. During this time, management consisted of IV hydration and transfusion. Across admissions, he received a total of 22 units of packed red blood cells. On his fifth admission, during which he received eight units of blood, he was administered an infusion of methylene blue for a methemoglobin level of 3.1%. This infusion was administered intravenously at a dose of 96.5 mg (dosing was 1 mg/kg in 50 mL D5W over 30 minutes). He improved and was discharged the following day and no hospitalizations or transfusions since. Hemoglobin and hematocrit 8 months after infusion were 9.3 g/dL and 34.5%, respectively—the highest

Table 1: Laboratory workup during August 2021 admission and the weeks following discharge

Test/Marker	Lab values	MB*	Lab values					Normal reference range
	8/4/21 20:37	8/4/21 23:28	8/5/21 05:17	8/6/21 11:29	8/9/21 12:08	8/12/21 13:24	8/20/21 16:55	
Bilirubin, total	2.8		2.5	2.9	3.3	3.2	…	0.0–1.0 mg/dL
Bilirubin, conjugated	0.7		0.7	0.8	0.8	0.6	…	0.0–0.4 mg/dL
LDH	489		426	465	329	328	316	105–333 U/L
Met-hemoglobin	12.0		7.8	…	12.9	13.3	14.3	0%–2%
Hemoglobin	11.2		9.4	11.2	10.9	9.9	9.8	Male: 13.4–17.5 g/dL
Hematocrit	36.8		31.7	39.0	36.0	33.5	33.1	Male: 38.9%–52.0%
Platelet count	582		506	563	584	552	602	130–400×10^3/uL
MCHC	30.4		29.7	28.7	30.3	29.6	29.6	31.0–37.0 g/dL
RDW-CV	25.7		25.3	24.4	24.0	25.6	23.9	12%–15%
Haptoglobin	<3		…	<3	…	<3	…	41–165 mg/dL

*Note: Methylene Blue was administered on 8/4/21 at 23:28.

values observed for this patient in over two years. To date, we have not taken a repeat methemoglobin value.

Discussion

Volga anemia is caused by a genetic defect that results in the replacement of non-polar, hydrophobic alanine with hydrophilic (at acidic pH) aspartic acid. This substitution alters the reduction/oxidation chemistry of the heme (iron, Fe) molecule of hemoglobin in a way that diminishes oxygen distribution potential and makes blood cells unstable through reactive oxygen species (ROS). As such, patients who possess this mutation develop both functional anemia from the inability of blood to let go of oxygen in peripheral tissues, and hemolytic anemia from premature cell lysis due to the ROS. Patients who carry this irregular hemoglobin typically fall into an anemic crisis when the body is undergoing insult or illness (i.e. ulcerative colitis flare, URI, dehydration from illness or activity). A potential cause is the nature of the aspartic acid substitution, as these states of physical insult can cause a change in pH which may increase the anemia's burden.

Other varieties of hemoglobin irregularities lead to methemoglobin production. One such anemia is from a gene substitution of tyrosine with histidine in what is known as the heme pocket of the genetic sequence this type is known as hemoglobin M.[8] Another type results from the deficiency of an enzyme needed in the body's natural reducing systems (i.e., cytochrome-b5 reductase (or methemoglobin reductase) or nicotinamide adenine dinucleotide phosphate (NADPH) reductase).[7] The reduction of methemoglobin by the body primarily occurs through the enzyme cytochrome b5 reductase. If this enzyme is underactive, overwhelmed by a massive build-up of methemoglobin, or rendered ineffective by a toxin, then the use of methylene blue to "unlock" an alternate pathway can be effective [Figure 1]. Of note, Patient A in this study underwent methemoglobin analysis in 2019, which showed a methemoglobin reductase level of 4.1 U/g Hb (normal 6.6–13.3 U/g Hb).

The enzyme NADPH-methemoglobin reductase is important in the treatment of methemoglobinemia.

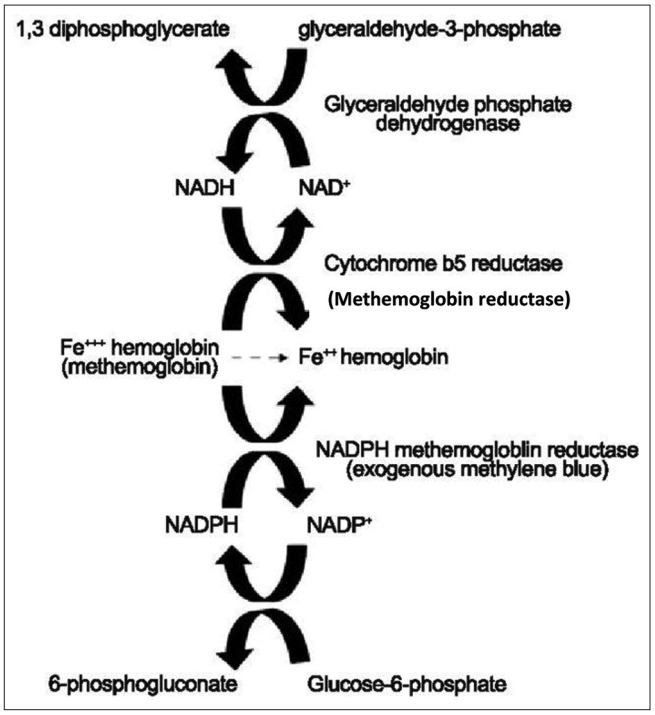

Figure 1: Metabolic pathways for the reduction of methemoglobin. The major pathway for methemoglobin reduction is cytochrome b5 reductase. An alternative pathway that requires an exogenous electron acceptor (i.e. methylene blue) is NADPH methemoglobin reductase. A small amount of methemoglobin is reduced through a nonenzymatic pathway (dashed line) 8

NADPH-methemoglobin reductase quickly reduces methemoglobin to ferrohemoglobin when exposed to methylene blue [Figure 1]. Methylene blue provides a diagnostic and therapeutic response, causing a rapid decrease in methemoglobin within 1–2 hours if the cause of the methemoglobinemia is due to a deficiency of methemoglobin reductase or a toxic agent. If one does not respond to methylene blue, it signifies that the methemoglobinemia is due to an M-type hemoglobin.[9] Since our patients had either a significant clinical or laboratory response with the methylene blue treatment for the Volga anemia crisis, this confers that the Volga mutation is not an M-type hemoglobin.

It is noteworthy that the patients in this series do not possess a G6PD deficiency. Known or suspected G6PD deficiency is a contraindication to the use of methylene blue because G6PD is the key enzyme in the formation of NADPH through the pentose phosphate pathway and G6PD. Deficient individuals generate insufficient NADPH to efficiently reduce methylene blue to leukomethylene blue, which is necessary for the activation of the NADPH-dependent methemoglobin reductase system. Administration of methylene blue to a patient with a G6PD deficiency may result in severe hemolysis and worsening anemia.[8]

More research is needed to better understand the exact pathophysiology of this condition. We felt it was important to raise awareness among other primary care physicians about this rare condition and even rarer treatment demonstrated to be safe and effective for quick reduction of methemoglobin levels and clinical improvement. Unfortunately, data are limited and the benefit of the IV medication outside a 24-hour window, if any, is unknown. If the impact is temporally limited, daily use of methylene blue is worth exploring. Daily IV administration is prohibitive, however, oral bioavailability has been shown to range from 53% to 97%.[1] Future studies of using oral methylene blue in patients with Volga anemia to achieve long-term reduction in methemoglobin levels would be informative.

Although Volga anemia is rare, we felt it was important to share this relatively unknown treatment for a hemolytic crisis using methylene blue with primary care providers to increase awareness and potentially decrease the time to diagnosis and intervention for future patients.

List of abbreviations

Abbreviation	Definition
IV	intravenous
mg	milligrams
kg	kilograms
Ala	alanine
Asp	aspartic acid
G6PD	glucose-6-phosphate dehydrogenase
E. coli	Escherichia coli
ICU	intensive care unit
g/dL	grams per deciLiter
LDH	lactate dehydrogenase
mg/dL	milligrams per deciLiter
DVT	deep vein thrombosis
SPO2	saturation of peripheral oxygen
mL	milliliters
D5W	dextrose (or glucose-5)
mg/kg	milligrams per kilogram
U/L	units per liter
U/g	Units per gram
pH	potential of hydrogen
ROS	reactive oxygen species
URI	upper respiratory infection
NADPH	nicotinamide adenine dinucleotide phosphate
Hb	hemoglobin

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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