Review Article

Methaemoglobinaemia: A Review

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Abstract: Methaemoglobin results from oxidation of the iron moieties in haemoglobin from the ferrous (Fe2+) to the ferric (Fe3+) state. Methaemoglobin levels in humans are maintained at 1-2% by the methaemoglobin reductase enzyme system. This enzyme reduces haemoglobin iron by transfer of an electron from NADH to oxidize cytochrome b5; cytochrome b5 then converts ferric iron to ferrous iron by direct interaction with haemoglobin. Methaemoglobinaemia occurs when the reductase enzyme system is overwhelmed and there is sustained elevated methaemoglobin in the circulation. Methaemoglobin is not compatible with life and should be prevented. It can be hereditary or acquired methaemoglobinaemia.

Keywords: Methaemoglobin, haemoglobin, ferrous, cytochrome.

INTRODUCTION

Normally, methaemoglobin levels are 1-2% using spectrophotometric method according to Dacie et al [1] in healthy humans. Elevated levels of methaemoglobin in the blood are caused when the mechanism that defend against oxidative stress within the red blood cells are overwhelmed and the oxygen carrying ferrous ion (Fe2+) of the haem group of the haemoglobin molecule is oxidized to the ferric state (Fe3+) according to Barker et al [2].

Methaemoglobin results from oxidation of the iron moieties in haemoglobin from the ferrous (Fe2+) to the ferric (Fe3+) state according to Hoffman [3]. Normal oxygenation of haemoglobin causes a partial transfer of an electron from the iron to the bound oxygen. Iron in this state thus resembles superoxides (O2). Deoxygenation returns the electron to the Iron, with release of oxygen. Methaemoglobin levels in humans are in fact maintained at < 1% by the methaemoglobin reductase enzyme system (nicotinamide adenine dinucleotide [NADH] - dehydratase, NADH - diaphorase, erythrocyte cytochrome b5 reductase). This enzyme reduces haemoglobin iron by transfer of an electron from NADH to oxidise cytochrome b5; cytochrome b5 then converts ferric to ferrous iron by direct interaction with haemoglobin. The generation of NADH depends on the glycolytic pathway [4].

A second reducing enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) - dependent methaemoglobin reductase, does not normally function in erythrocytes because no electron carrier is available in erythrocytes to interact with NADPH. Exogenous electron carriers, such as methylene blue, can provide the missing activity. Agents such as methylene blue, can therefore serve as pharmacologic agents for the treatment of methaemoglobinemia. "Reduced glutathione and ascorbic acid reduce, methaemoglobin directly, but these nonenzymatic reactions are considerably slower than the reductase pathways [5,6].

DEFINITION AND HISTORY OF METHAEMOGLOBINEMIA

A bluish discolouration of the skin and mucous membranes, designated cyanosis, has been recognized since antiquity as a manifestation of lung or heart disease. Cyanosis resulting from drug administration has also been recognized since before 1890 [7]. Toxic methaemoglobinemia occurs when various drugs or toxic substances either oxidize haemoglobin directly in the circulation or facilitates its oxidation by molecular oxygen.

In 1912 Sloss and Wybauw reported a case of a patient with idiopathic methaemoglobinemia [8]. Later, Hitzenberger [9] suggested that a hereditary form of methaemoglobinemia might exist, and subsequently numerous such cases were reported 10. In 1948
Horlein and Weber described a family in which eight members over four generations manifested cyanosis. The absorption spectrum of methaemoglobin was abnormal. They demonstrated that the defect must reside in the globin portion of the molecule. Subsequently Singer suggested that such abnormal haemoglobins be given the designation haemoglobin M [11].

The cause of still another form of methaemoglobinemia that occurs independent of drug administration and without the existence of any abnormality of the globin portion of haemoglobin was first explained by Gibson [6], who clearly pointed to the site of the enzyme defect, NADH diaphorase.

**TYPES AND CAUSES OF METHAEMOGLOBINEMIA**

**Congenital Methaemoglobinemia:** The congenital form of methaemoglobinemia has an autosomal recessive pattern of inheritance. Due to a deficiency of the enzyme diaphorase 1 (NADH methaemoglobin reductase), methaemoglobin levels rise and the blood of methaemoglobin patients has reduced oxygen-carrying capacity. Instead of being red in colour, the arterial blood of Met - Hb patients is brown. This results in the skin of Caucasian patients gaining a bluish hue. Hereditary MetHb is caused by a recessive gene. If only one parent has the gene, offspring will have normal - hued skin, but, if both parents carry the gene there is a chance the offspring will have blue - hued skin.

Another cause of congenital methaemoglobinemia is seen in patients with abnormal haemoglobin variants such as haemoglobin M (HbM), or haemoglobin H (HbH), which are not amenable to reduction despite intact enzyme systems. Methaemoglobinemia can also arise in patients with pyruvate kinase deficiency due to impaired production of NADH - the essential cofactor for diaphorase I. Similarly, patients with Glucose - 6 - phosphate dehydrogenase (G6PD) deficiency may have impaired production of another co-factor, NADPH.

**Acquired Methaemoglobinemia:** Methaemoglobinemia can also be acquired [12]. The protective enzyme systems normally present in red blood cells maintain methaemoglobin levels at less than one percent of the total haemoglobin in healthy people. Exposure to exogenous oxidizing drugs and their metabolites (such as benzocaine, dapsone, and nitrates) may accelerate the rate of formation of methaemoglobin up to one - thousand fold, overwhelming the protective enzyme systems and acutely increasing methaemoglobin levels. Other classical drug causes of methaemoglobinemia include antibiotics (trimethoprin, sulphonamides and dapsone) [13], local anaesthetics (especially articaine and prilocaine) as opined by Adams et al [14], and others such as aniline dyes, metoclopramide, chlorates and bromates. Ingestion of compounds containing nitrates can also cause methaemoglobinemia.

Infants under 6 months of age are particularly susceptible to methaemoglobinemia caused by nitrates ingested in drinking water (Called blue - baby syndrome), dehydration usually caused by gastroenteritis with diarrhea, sepsis and tropical anaesthetics containing benzocaine or prilocaine. Nitrates that are used in agricultural fertilizers leaked into the ground and many contaminate well water. The current Environmental protection Agency (EPA) standard of 10ppm nitrate - nitrogen for drinking water is specifically designed to protect infants [15].

**CARRIERS OF METHAEMOGLOBINEMIA**

The Fugates, a family that lived in the hills of Kentucky, are the most famous example of this hereditary genetic error, known as the Blue Fugates, Martin Fugate, settled near Hazard, Kentucky, Circa 1800. His wife was a carrier of the recessive methaemoglobinemia (MetHb) gene, as was a nearby clan with whom the Fugates intermarried. As a result, many descendants of the Fugates were born with MetHb according to straight Dope Article on Fugates of Appalachia, an extended family of blie skinned people.

The ‘blue men of Lurgan’ were a pair of Lurgan men suffering from what was described as “familial idiopathic methaemoglobinemia” who were treated by Dr. James Deeny in 1942. Denny who would later become the Chief Medical Officer of the Republic of Ireland, prescribed a course of ascorbic acid and sodium bicarbonate. In case one, by the eight day of treatment there was a marked change in appearance and by the twelfth day of treatment the patient’s complexion was normal. In case two, patients complexion reached normality over a month - long duration of treatment [16].

**PATHOGENESIS AND CLINICAL MANIFESTATIONS**

Methaemoglobinemias of clinical interest arise by one of three distinct mechanisms:

1. Globin chain mutations that result in increased formation of methaemoglobin.
2. Deficiencies of methaemoglobin reductase and
3. Toxic methaemoglobinemia, in which normal red cells, are exposed to substances that oxidize haemoglobin iron such that normal reducing mechanisms are subverted or overwhelmed.

Abnormal haemoglobins producing methaemoglobinemia (HbM) arise from mutations that stabilize the haeme iron in the ferric state. Classically, a histidine in the vicinity of the haeme pocket is replaced by a tyrosine; the hydroxyl group of the tyrosine forms a complex that stabilizes the iron in the ferric state. The
oxidized haeme iron is relatively resistant to reduction by the methaemoglobin reductase system [4].

Sign and symptoms of methaemoglobinemia (>1%) include shortness of breath, cyanosis, mental

Severe, methaemoglobinemia (>50%) patients have dysrhythmias, seizures, coma and death (>70%). Healthy people may not have symptoms methaemoglobin levels ≤15%, however patients with co-morbidities such as anaemia, cardiovascular disease, lung disease, sepsis, or presence of other abnormal hemoglobin species (carboxyhaemoglobin, sulphhaemoglobin or sickle haemoglobin) may experience moderate to severe symptoms at much lower levels (as low as 5-8%).

Hereditary methaemoglobinemia resulting from methaemoglobin reductase deficiency is very rare. Numerous recessive mutations cause a variety of abnormalities of enzymatic activity or amount, including catalytic activity, electrophoretic mobility, and structural stability. Some individuals exhibit neurological defects. The mutation might thus affect isoforms of the enzyme common to 'both erythrocyte and other tissues, including brain [17].

Like patients with M haemoglobins, patients with methaemoglobin reductase deficiency exhibit slight gray "pseudo-cyanosis". Even homozygotes; however, rarely accumulate > 25% methaemoglobin, a level compatible with absence of symptoms. Heterozygotes can have normal methaemoglobin levels but are especially sensitivity to agents that cause methaemoglobinemia.

A third toxic form of methaemoglobinemia caused by exposure to certain chemical agents and drugs that accelerate the oxidation of methaemoglobin. Nitrite compounds are especially notorious and common. Some of these compounds also have a propensity to exacerbate G6PD deficiency and exacerbate the precipitation of unstable haemoglobins. Nitrates are frequent environmental cause of toxic methaemoglobinemia. Nitrates do not directly interact with either haemoglobin or the reductase pathway but are converted to nitrites in the gut. Well water is a frequently encountered source of excessive nitrates. In general, substantial, intake of these agents is required before significant amounts of methaemoglobin are generated. Very young infants are more susceptible to these agents than are adults, but all age groups are at risk given sufficient exposure [4].

**HOSPITALIZATION STATISTICS FOR METHAEMOGLOBINEMIA**

The following are statistics from various sources about hospitalization and methaemoglobinemia. 0.0001% [10] of hospital consultant episodes were for status change (-50%), headache, fatigue, exercise intolerance, dizziness and loss of consciousness. Arterial blood with elevated methaemoglobin levels has a characteristic chocolate - brown colour as compared to normal bright red oxygen containing arterial blood. Methaemoglobinemia in England 2002-2003[18]. 100% of hospital consultant episodes for methaemoglobinemia required hospital admission in England 2002 – 2003[18]. 60% of hospital consultant episodes for methaemoglobinemia were for men in England 2002-2003[18]. 40% of hospital consultant episodes for methaemoglobinemia were for women in England 2002-2003. 60% of hospital consultant episodes for methaemoglobinemia required emergency hospital admission in England 2002 – 2003[18]. 12 days was the mean length of stay in hospital for methaemoglobinemia in England 2002-2003. 0 days was the median length of stay in hospitals for methaemoglobinemia in England 2002 - 2003. 15 were the mean age of patients hospitalized for methaemoglobinemia in England 2002-2003[18]. 40% of hospital consultant episodes for methaemoglobinemia occurred in 15 -59 years old in England 2002 – 2003[18]. 0% of hospital consultant episodes for methaemoglobinemia occurred in people over 75 in England 2002 – 2003[18]. 10% of hospital consultant episodes for methaemoglobinemia were single day episodes in England 2002 – 2003[18]. 0% [11] of hospital bed days were for methaemoglobinemia in England 2002 -2003[18].

**DIAGNOSIS OF METHAEMOGLOBINEMIA (Hi)**

The diagnosis is done using spectrophotometric method according to Dacie et al [1].

**Method**

Lyze 0.2ml of blood in a solution containing 4ml of buffer and 6ml of detergent solution. Divide the lysate into two equal volume (A and B). Measure the absorbance of A in a spectrophotometer at 630nm (Di).

Add 1 drop of potassium cyanide solution to B, and after 5mins, measure the absorbance again, after mixing (D2). Then add 1 drop of potassium ferricyanide solution to B, after 3mins, measure the absorbance at the same wavelength (D3).

Hi(%) = \frac{D1-D2}{D3-D4} \times 100

**TREATMENT OF METHAEMOGLOBINEMIA**

Methaemoglobinemia can be treated with supplemental oxygen and methylene blue 15]. 1% solution (10mg/ml) 1-2mg/kg administered intravenously slowly over 5 minutes followed by intravenous flush with normal saline. Methylene blue restores the iron in haemoglobin to its normal (reduced) oxygen - carrying state. This is achieved through the enzyme including effect of methylene blue on levels of
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CONCLUSION

A bluish discolouration of the skin and mucous membranes, designated cyanosis, has been recognized since antiquity as a manifestation of lung or heart disease. Cyanosis resulting from drug administration has also been recognized. Toxic methaemoglobinemia occurs when various drugs or toxic substances either oxidize haemoglobin directly in the circulation or facilitates its oxidation by molecular oxygen. Infants under 6 months of age are particularly susceptible to methaemoglobinemia caused by nitrate ingested in drinking water (Called blue - baby syndrome), dehydration usually caused by gastroenteritis with diarrhea, sepsis and tropical anaesthetics containing benzocaine or prilocaine. Nitrates that are used in agricultural fertilizers leaked into the ground and many contaminate well water. Methaemoglobinemia should be avoided because of its lethality to life. Methaemoglobinemia can be treated with supplemental oxygen, rehydration and methylene blue.

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