Case report

A female patient inherits congenital methemoglobinemia due to a novel homozygous CYB5R3 mutation and G6PD deficiency

Natwipha Ratchaneewong[1], Chattree Hantaweepant[1], Chanin Limwongse[2], Sitanun Preechathaveekid[1], Nawapotch Donsakul[1], Pongthep Vittayawacharin[1], Jane Jianthanakanon[1], Tarinee Rungjirajittranon[1], Smith Kungwankiattichai[1], Chutima Kunacheewa[1], Weerapat Owattanapanich[1], Ployploen Phikulsod[1], Ekapun Karoopongse[1], Bundarika Suwanawiboon[1], Archrob Khuhapinant[1], Yingyong Chinthammitr[1] and Theera Ruchutrakool[1]

[1]Division of Hematology; [2]Division of Medical Genetics, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University

Abstract:

Methemoglobinemia is a disorder that can result from inherited or acquired etiologies. Congenital methemoglobinemia, being less common, can occur from autosomal recessive mutations in the enzyme cytochrome b5 reductase (CYB5R) or autosomal dominant mutations in the globin genes. We present a case of a 32-year-old woman with a lifelong history of cyanosis experiencing dyspnea for 5 hours after consuming cured sausages and dyeing her hair. She was admitted to hospital due to acute respiratory failure with an oxygen saturation of 80%. Physical examination revealed central and peripheral cyanosis. Arterial blood gas analysis showed a PaO_2 of 292 mmHg and SaO2 of 100%. The calculated oxygen saturation gap was 20%. Methemoglobin level was elevated at 36.9%. Other laboratory findings and peripheral blood smear were consistent with acute intravascular hemolysis from oxidative stress. The result of flow cytometry for glucose-6-phosphate dehydrogenase (G6PD) was compatible with G6PD deficiency. Direct DNA sequencing analysis identified a novel homozygous mutation, likely a pathogenic missense variant in the CYB5R3 gene, c.802_803delGAinsCC (p.Glu286Pro). The diagnosis in this patient encompasses acquired methemoglobinemia superimposed on congenital methemoglobinemia and acute hemolysis from G6PD deficiency. Because methylene blue is contra-indicated among patients with G6PD deficiency, treatment with N-acetylcysteine and supportive measures were initiated, resulting in symptom improvement in this patient.

Keywords : ● *Congenital methemoglobinemia* ● *CYB5R3 mutation* ● *CYB5R deficiency* ● *Central cyanosis* ● *Oxygen saturation gap*

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Correspondence should be addressed to Chattree Hantaweepant, MD., Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University Bangkok 10700

รายงานผู้ป่วย

ผู้ป่วยเพศหญิงที่มีภาวะเมธฮีโมโกลบินนีเมียทางพันธุกรรมจากการขาดเอนไซม์ Cytochrome B 5 Reductase และภาวะเม็ดเลือดแดงแตกจากการขาดเอนไซม์ G6PD

ณัฐวิภา รัชนีวงศ์[1] ฉัตรี หาญทวีพันธุ์[1] ชนินทร์ ลิ่มวงศ์[2] สิตานัน ปรีชาทวีกิจ[1] นวพชร ดอนสกุล[1]
พงษ์เทพ วิทยาวัชรินทร์[1] เจน เจียรธนะกานนท์[1] ธาริณี รุ่งจิรจิตรานนท์[1] สมิทธ์ กังวานเกียรติชัย[1] ชุติมา คุณาชีวะ[1]
วีรภัทร โอวัฒนาพานิช[1] พลอยเพลิน พิกุลสด[1] เอกพันธ์ ครุพงศ์[1] บุณฑริกา สุวรรณวิบูลย์[1] อาจรบ คูหาภินันทน์[1]
ยิ่งยง ชินธรรมมิตร์[1] และ ธีระ ฤชุตระกูล[1]
[1]สาขาวิชาโลหิตวิทยา [2]สาขาวิชาเวชพันธุศาสตร์ ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

บทคัดย่อ

ภาวะเมธฮีโมโกลบินนีเมีย (methemoglobinemia) หมายถึง ภาวะที่กระแสโลหิตมีระดับความเข้มข้นของเมธฮีโมโกลบิน (methemoglobin) มากกว่าปกติ ซึ่งอาจทำให้เนื้อเยื่อและอวัยวะภายในร่างกายเกิดภาวะขาดออกซิเจน จนอาจเป็นอันตรายต่อชีวิตได้ ภาวะนี้สามารถเกิดจากการได้รับยาหรือสารบางอย่าง หรือเป็นจากโรคทางพันธุกรรม คณะผู้รายงานได้รายงานผู้ป่วยหญิงไทย อายุ 32 ปีมาด้วยอาการเหนื่อย 5 ชั่วโมงหลังรับประทานไส้กรอกและย้อมผม ตรวจร่างกายพบภาวะเขียว เครื่องวัดความอิ่มตัวของออกซิเจนที่ปลายนิ้ว (pulse oximetry) วัดได้ค่า 80 เปอร์เซ็นต์ ผลตรวจก๊าซจากหลอดเลือดแดง (arterial blood gas) พบค่าออกซิเจนในเลือดแดงอยู่ในระดับปกติ ระดับเมธฮีโมโกลบินในเลือดวัดได้ 36.9 เปอร์เซ็นต์ และพบภาวะเม็ดเลือดแดงแตกในหลอดเลือดจากการขาดเอนไซม์ glucose-6-phosphate dehydrogenase (G6PD) การศึกษาลำดับพันธุกรรมพบการกลายพันธุ์ในยีน CYB5R3 (c.802_803delGAinsCC, p.Glu286Pro) ซึ่งไม่เคยมีการรายงานมาก่อน เนื่องจากยา methylene blue ใช้ไม่ได้ผล และอาจทำให้ภาวะเม็ดเลือดแดงแตกและภาวะเมธฮีโมโกลบินนีเมียแย่ลงในผู้ป่วยที่ขาดเอนไซม์ G6PD ผู้ป่วยรายนี้ได้รับการรักษาภาวะเมธฮีโมโกลบินนีเมีย ด้วยยา N-acetylcysteine และการรักษาแบบประคับประคองจนอาการดีขึ้นในที่สุด

คำสำคัญ : ● *Congenital methemoglobinemia* ● *CYB5R3 mutation* ● *CYB5R deficiency* ● *Central cyanosis* ● *Oxygen saturation gap*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2567;34:.139-47.

Introduction

Methemoglobinemia is a rare disease associated with oxidation of ferrous iron (Fe^{2+}) in hemoglobin (Hb) to ferric iron (Fe^{3+}) of methemoglobin (MetHb), which is unable to bind and carry oxygen effectively. Therefore, a substantial increase in MetHb levels results in heightened oxygen affinity of the remaining Hb containing ferrous iron, consequently hindering the release of oxygen to the tissue, leading to hypoxia.[1] Methemoglobinemia can stem from inherited or acquired factors, with acquired forms being more prevalent and arising from exposure to oxidizing substances, i.e., some drugs or chemical agents accounting for the acceleration of iron in Hb from the ferrous to the ferric state. Congenital forms are rare, resulting from autosomal recessive defects in the CYB5R3 gene known as recessive congenital methemoglobinemia (RCM), which causes an enzyme deficiency for MetHb reduction[2], or they can be caused by an autosomal dominant mutation in the globin genes, known as Hb M disease. Structural abnormalities of globins result in auto-oxidation of Hb to MetHb.[1] We present a case of congenital methemoglobinemia caused by a novel homozygous mutation in the CYB5R3 gene.

Case presentation

A 32-year-old female presented at an emergency department with dyspnea for 5 hours. Twelve hours prior, she had consumed 10 to 15 cured sausages and then had dyed her hair. During hair dyeing process, she felt fatigued and had mild chest discomfort. Afterwards, she developed acute progressive dyspnea. She denied any history of fever, cough, or chest pain. On physical examination, she was afebrile with a body temperature of 37°C, blood pressure of 120/74 mmHg, pulse rate of 87 beats/minute, respiratory rate of 30 breaths/minute, and oxygen saturation of 80% by pulse oximetry. The patient was fully conscious, exhibiting central and peripheral cyanosis (Figure 1). She showed no signs of pallor, jaundice, edema, or finger clubbing. Respiratory and cardiovascular examinations were normal, and other physical examinations were unremarkable.

The initial arterial blood gas (ABG) analysis revealed an arterial oxygen saturation (SaO_2) of 100%, pH of 7.4, partial pressure of oxygen (PaO_2) of 292 mmHg, partial pressure of carbon dioxide ($PaCO_2$) of 24 mmHg, and bicarbonate (HCO_3) of 14 mmol/L. The chest X-ray was normal. Electrocardiogram showed a sinus tachycardia with a rate of 100/min, normal axis, normal PR and QT intervals, no chamber enlargement, non-specific ST-T change, and no S1Q3T3. The complete blood count (CBC) revealed a Hb of 16.0 g/dL, hematocrit (Hct) of 51%, mean corpuscular volume (MCV) of 88.4 fL, mean corpuscular hemoglobin (MCH) of 27.8 pg, mean corpuscular hemoglobin concentration (MCHC) of 31.5 g/dL, red cell distribution width (RDW) of 15.9%, white blood cell (WBC) count of 6,900 and platelet count of 202,000/μL. Liver function tests (LFTs) showed a total bilirubin (TB) of 3.2, indirect bilirubin (IB) of 2.1 mg/dL, aspartate aminotransferase (AST) of 31, alanine aminotransferase (ALT) of 15, and alkaline phosphatase (ALP) of 62 U/L. The creatinine level was 0.47 mg/dL. The electrolyte tests showed a sodium of 138, potassium of

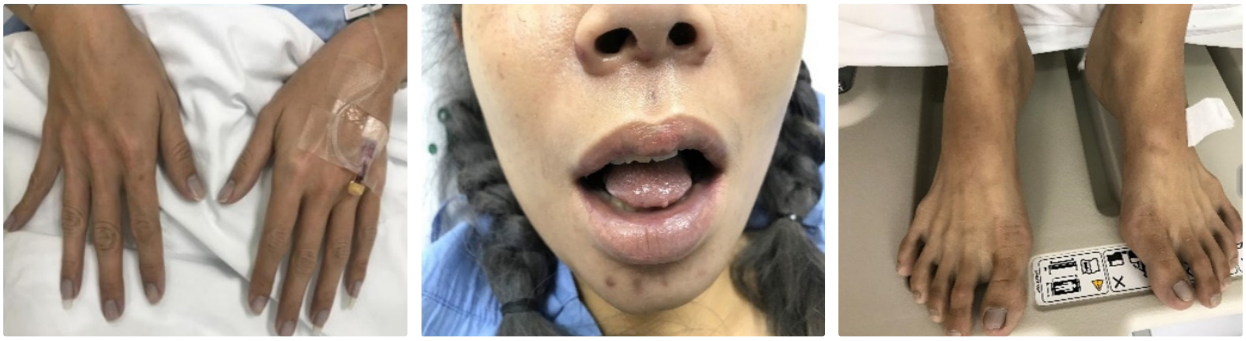

Figure 1 The patient's central and peripheral cyanosis

4, chloride of 105, and bicarbonate of 18 mmol/L. Serum lactate dehydrogenase level (LDH) was 255 U/L (female normal range 135-214), and lactate was 4 mmol/L. Urinary analysis revealed occult blood 2+, red blood cell (RBC) count > 100/high power field, WBC 20-30/high power field, bilirubin 1+, and urobilinogen 1+.

She was admitted to an internal medicine ward, and a diagnosis of methemoglobinemia was considered based on the oxygen saturation gap of 20%. An oxygen saturation gap represents a difference of more than 5% between the oxygen saturation from an ABG and a reading from pulse oximetry. The MetHb level could not be measured initially due to a limitation in laboratory capacities at the primary hospital. She was initially managed with an oxygen mask with reservoir bag, delivering oxygen at 10 liters per minute. Nevertheless, her oxygen saturation rose only slightly from 80 to 85%. Six hours later, her symptoms worsened, and her oxygen saturation dropped to 75%, leading to respiratory failure. She was intubated with an endotracheal tube and received mechanical ventilation set at a fraction of inspired oxygen (FiO2) of 1.0. An ABG showed an SaO_2 of 100%, pH of 7.56, PaO_2 of 606.9 mmHg, $PaCO_2$ of 22.6 mmHg, and HCO_3 of 18 mmol/L while pulse oximetry still showed an oxygen saturation of 88%. Subsequently, she was referred to our tertiary center, Siriraj Hospital, for further investigation and management. Upon physical examination, she presented fever with a body temperature of 38°C. Other physical examination findings were consistent with those observed at the previous hospital except that her urine color was dark.

The level of MetHb measured by the spectrophotometric method at 23 hours after the onset of symptoms was 36.9%. Her CBC showed a decrease of Hb from 16.0 to 12.6 g/dL and Hct from 51 to 38.6% with normal WBC and platelet counts. A peripheral blood smear revealed normochromic normocytic RBCs, increased polychromasia along with blister cells, bite cells and ghost cells (Figure 2). The absolute reticulocyte count was 232,360/μL. LFTs showed a TB of 6.36, IB of 6 mg/dL, AST 18, ALT 11, and ALP 51 U/L. The LDH was 224 U/L, and haptoglobin was 22.6 mg/dL (normal range 30-200). Her medical history was obtained from records provided by her mother. At the age of 1 year, her mother noticed a bluish discoloration of her lips and skin along with increased breathlessness compared with other children while she was playing. She had no other symptoms, and her developmental milestones were normal. At the age of 9 years, she was brought to the hospital to investigate the cause of the bluish discoloration. The pediatrician observed central and peripheral cyanosis. Cardiac and pulmonary evaluation were normal. Due to the unexplained cyanosis and hypoxemia, methemoglobinemia was suspected, and the MetHb level was found to be 45.6%. Eventually, she received a diagnosis of congenital methemoglo-

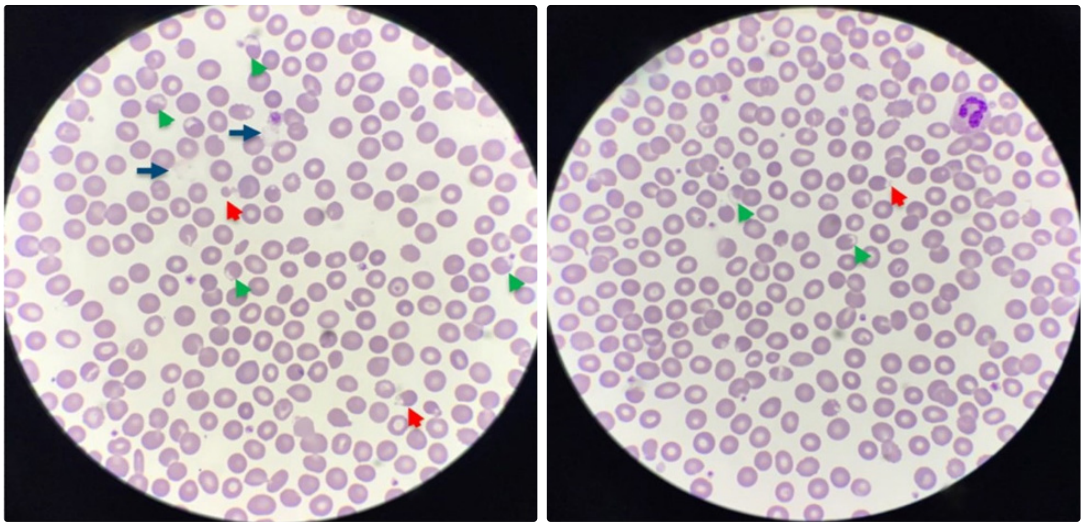

Figure 2 Patient's peripheral blood smear demonstrating blister cells, bite cells, and ghost cells

binemia and G6PD deficiency. She did not receive any treatment and was lost to follow up. In adulthood, she visited hospitals multiple times for medical checkups, and her oxygen saturation ranged from 78 to 85% on room air during each visit.

During the present admission, she was suspected of acquiring methemoglobinemia in addition to congenital methemoglobinemia. The acquired cause was attributed to oxidative stress, potentially from potassium nitrite, a food preservative used to maintain the red color of meat in the cured sausages, combined with aniline in the hair dye. Although her MetHb level could not be measured at the onset of symptoms, it was assumed to be higher than 36.9%. Consequently, she was admitted to the intensive care unit. Consultations with hematologists and toxicologists were sought. A septic workup was also conducted to identify the source of her fever, revealing a urinary tract infection (UTI). Due to the decrease in Hb, acute intravascular hemolysis from G6PD deficiency was considered as the most likely diagnosis based on the fact that she had dark urine, reticulocytosis, indirect hyperbilirubinemia and low haptoglobin in conjunction with the presence of blister cells, bite cells and ghost cells in the peripheral blood smear. Flow cytometry for G6PD was used to confirm G6PD deficiency.[3] This method used DNA analysis for G6PD gene mutation as a gold standard. It can determine heterozygous or homozygous G6PD deficiency among females or hemizygote among males. The result is presented with the percentage of red blood cells with normal G6PD levels (bright cells). Among female patients, bright cells ranging from 85.4 to 100%, 6.3 to 85.3%, and 0 to 6.2%, are interpreted as individuals with no G6PD deficiency, with heterozygous deficiency and with homozygous deficiency, respectively. The patient's result demonstrated 36.2% of bright cells, which was consistent with heterozygous G6PD deficiency. The hemolytic triggers could have been the UTI and aniline in hair dye, which is a recognized oxidative substance.

She was managed with ventilatory support, antibiotics and hydration. Methylene blue (MB) is the first-line agent for treating symptomatic methemoglobinemia. However, this is contraindicated among patients with G6PD-deficiency. Firstly, MB is an oxidative substance, so it can induce both methemoglobinemia and hemolysis among patients with G6PD-deficiency. Secondly, MB requires nicotinamide adenine dinucleotide phosphate (NADPH), which is produced by the G6PD enzyme to reduce MetHb to Hb. Therefore, MB lacks efficacy among patients with G6PD deficiency. Consequently, we decided to administer N-acetylcysteine (NAC) at a dosage of 300 mg/kg/day for 5 days as an anti-oxidant instead. In this case, the precipitating factors were potassium nitrite and aniline dye. We expected that the oxidative substances should be excreted within 24 hours, considering the half-lives of MetHb, potassium nitrite, and aniline, which are 1-3 hours, 4-8 hours, and 6 hours, respectively. One day later, her symptoms improved, and she was successfully extubated. Her oxygen saturation at room air and metHb level were around 80-85% and 36-38%, respectively. Antibiotic treatment led to the resolution of fever. Additionally, a follow-up CBC showed that her Hb increased to 14 g/dL, and the hemolysis was resolved. She stayed in hospital for 4 days and was safely discharged without further medication or treatment.

Due to a lifelong history of cyanosis and persistently high levels of MetHb, congenital methemoglobinemia was suspected. To determine whether she has Hb M disease or CYB5R deficiency, Hb electrophoresis was performed, revealing the proportions of 75% Hb A, 21.9% Hb E, and 3.1% HbA_2. Direct DNA sequencing of alpha and beta globin genes showed normal alpha globins with Hb E heterozygote. Thus, Hb M disease was excluded. Mutation analysis of the CYB5R3 gene was performed by direct sequencing of the entire coding sequencing of the CYB5R3 gene using b5r-F1/b5r-R1 primers for cDNA amplification of 909 bp fragment.[4] The result revealed a deletion of GA with insertion of CC nucleotides at

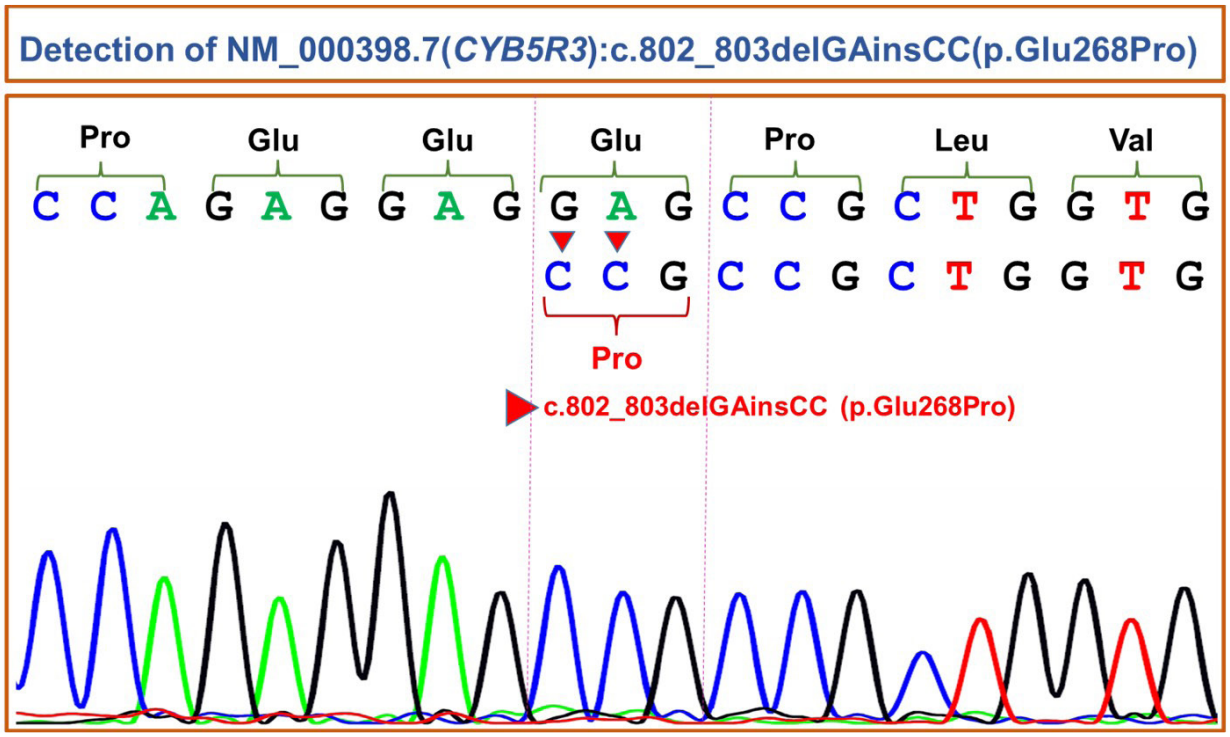

Figure 3 Sequencing chromatogram in exon 9 of the CYB5R3 gene showing a deletion of GA replaced by an insertion of CC at the nucleotide position 802_803, resulting in substitution of glutamic acid to proline at codon 268 (c.802_803delGAinsCC, p.Glu286Pro)

position 802_803 in exon 9, which is a novel homozygous mutation resulting in the substitution of glutamic acid to proline at codon 268 (c.802_803delGAinsCC, p.Glu286Pro) (Figure 3). This variant was predicted to be novel and likely pathogenic based on the published classification criteria of the 2015 American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) standards and guidelines (PS3, PM2_strong, PP2).[5] PS3 criteria (strong evidence of pathogenicity) was given because the clinical finding of elevated methemoglobinemia is considered in vivo confirmation of pathogenicity. PM2_strong criteria (moderate evidence of pathogenicity) was classified because this variant was not found in control individuals in all available databases. PP2 criteria (supporting evidence of pathogenicity) was classified because it constitutes a missense CYB5R3 variant which is a common mechanism of congenital methemoglobinemia. Therefore, the presence of this homozygous likely pathogenic missense variant in the CYB5R3 gene contributes to defining this patient as congenital methemoglobinemia.

In the follow-up, she remained in good condition, occasionally experiencing fatigue with mild dyspnea. Her oxygen saturation from pulse oximetry and MetHb levels persisted at 84-90% and 36-38%, respectively. The management for this patient involved avoiding precipitating factors for both methemoglobinemia and G6PD deficiency.

Discussion

This case presents several intriguing issues. Firstly, it was diagnosed as RCM due to a novel homozygous mutation in the CYB5R3 gene. Secondly, the methemoglobin reduction technique by flow cytometry for G6PD could be used to diagnose G6PD deficiency in a patient with methemoglobinemia. Lastly, it highlights the challenge of managing symptomatic acquired methemoglobinemia among patients with G6PD deficiency.

Congenital methemoglobinemia results from either autosomal recessive mutations in the CYB5R3 gene or autosomal dominant variants in the globin genes. Our patient experienced a lifelong history of cyanosis since

childhood even in the absence of a positive family history. Thus, autosomal recessive inheritance was suspected. The diagnosis of methemoglobinemia due to CYB5R deficiency includes the measurement of cytochrome b5 reductase enzymatic activity at usually less than 20% of the normal level[1] and DNA analysis of the CYB5R3 gene. In our case, although enzymatic levels were not measured, a novel homozygous missense variant in the CYB5R3 gene, c.802_803delGAinsCC, p.Glu286Pro, supported a diagnosis of congenital methemoglobinemia.

RCM is caused by biallelic mutations in the CYB5R3 gene, containing 9 exons located on chromosome 22.[2] To date, more than 80 different disease-causing variants in the CYB5R3 gene have been reported.[6,7,8] The CYB5R3 gene encodes for NADH-dependent methemoglobin reductase or cytochrome b5 reductase enzyme (CYB5R). The NADH-dependent-CYB5R enzyme is the major enzyme involved in 95% of the process of MetHb reduction whereas NADPH-dependent methemoglobin reductase is less significantly involved in the remaining percentage of the process.[9] Therefore, CYB5R deficiency leads to methemoglobinemia.

CYB5R deficiency can be classified in two subtypes based on the severity of enzyme deficiency and differing patient phenotypes. RCM type I, primarily due to missense variants, a nucleotide change contributing to encoding a different amino acid, leads to the production of an unstable enzyme restricted to only the RBCs. Patients are mostly asymptomatic, but present symptoms including cyanosis, headache, fatigue and dyspnea. Patients typically have a normal life expectancy.[10] RCM type II, caused by nonsense mutations, deletions, and specific missense mutations that disrupt splicing or truncate the protein, results in either low expression or low activity of the enzyme in all cells. This type is associated with alterations in lipid metabolism and neurologic involvement, leading to severe progressive neurologic impairment. Patients with RCM type II have a short life expectancy primarily due to swallowing difficulties and respiratory complications with death typically occurring in the first decade of life.[1,10] Thus, our patient's phenotype was more compatible with RCM type I.

Flow cytometry for G6PD demonstrated that the patient had heterozygous G6PD deficiency, as this technique can detect the fluorescent activity of the methemoglobin reduction (MR) test within each RBC.[3] The MR test principle involves initially oxidizing all Hb to MetHb, which is then further reduced to Hb by NADPH-dependent methemoglobin reductase. This reduction process relies on NADPH, synthesized by G6PD. Therefore, RBCs among patients with G6PD-deficiency cannot effectively reduce MetHb, leading to high residual MetHb levels at the end of the reaction. These RBCs with high MetHb levels do not fluoresce, resulting in a lower percentage of bright cells (RBCs with normal G6PD activity) that can be interpreted as G6PD deficiency. As mentioned above, the MR test uses NADPH-dependent methemoglobin reductase to reduce MetHb *in vitro*. Therefore, the MR test can be accurately interpreted among patients with methemoglobinemia from CYB5R (NADH-dependent methemoglobin reductase) deficiency.

The management of methemoglobinemia in our patient can be addressed in two parts. The treatment of the acute episode precipitated by oxidants, and the treatment of congenital methemoglobinemia. The management of methemoglobinemia during an acute episode involved removing the precipitating factors and prescribing medication to symptomatic patients. Symptoms of methemoglobinemia depend on MetHb levels and the patient's pre-existing health status. Patients usually present hypoxic symptoms when MetHb levels are more than 30% whereas patients with comorbidities compromising oxygen delivery, such as heart diseases, lung diseases, or significant anemia, can develop symptoms with MetHb levels ranging from 10 to 30%.[1] The first-line drug is intravenous MB. The mechanism of action of MB, a pro-drug, involves its conversion to leukomethylene blue

by NADPH methemoglobin reductase. Leukomethylene blue reduces MetHb to oxyhemoglobin. However, MB should be avoided among patients with G6PD deficiency because it has oxidant potential that induces both hemolysis and worsens methemoglobinemia.[11] Moreover, MB is ineffective in such patients because they lack NADPH, which is produced by the G6PD enzyme and is required for NADPH methemoglobin reductase to reduce MB to leukomethylene blue.[12,13] Alternative treatments such as ascorbic acid, NAC or exchange transfusion in severe cases should be considered.[1,11] Our patient was treated with NAC. Evidence from *in vitro* studies have demonstrated that NAC can reduce MetHb levels in human blood with and without G6PD enzyme deficiency.[14,15]

Treatment of congenital methemoglobinemia primarily involves avoiding oxidizing substances because these patients are susceptible to developing acquired methemoglobinemia. Patients with RCM type I are typically asymptomatic except for cyanosis. Treatment is unnecessary. If the patient requires treatment only to improve a bluish appearance, the benefits and risks of drugs such as MB, ascorbic acid, or riboflavin should be discussed.[1] These drugs are ineffective in RCM type II.[16] In methemoglobinemia associated with Hb M disease, MB and ascorbic acid treatment are ineffective and should be avoided.[17]

Conclusion

Congenital methemoglobinemia is a rare genetic disorder that should be considered among patients with a lifelong history of cyanosis, even in the absence of a family history of the condition. Patients typically present central and peripheral cyanosis, which often does not respond to oxygen therapy and a wide oxygen saturation gap. G6PD deficiency should be ruled out before initiating treatment with methylene blue among patients with symptomatic acquired methemoglobinemia.

References

1. *Iolascon A, Bianchi P, Andolfo I, Russo R, Barcellini W, Fermo E, et al. Recommendations for diagnosis and treatment of methemoglobinemia. Am J Hematol. 2021;96:1666-78.*
2. *Percy MJ, Lappin TR. Recessive congenital methaemoglobinaemia: cytochrome b(5) reductase deficiency. Br J Haematol. 2008;141:298-308.*
3. *Thedsawad A, Wanachiwanawin W, Taka O, Hantaweepant C. Cut-off values for diagnosis of G6PD deficiency by flow cytometry in Thai population. Ann Hematol. 2022;101:2149-57.*
4. *Wu YS, Huang CH, Wan Y, Huang QJ, Zhu ZY. Identification of a novel point mutation (Leu72Pro) in the NADH-cytochrome b5 reductase gene of a patient with hereditary methaemoglobinaemia type I. Br J Haematol. 1998;102:575-7.*
5. *Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-24.*
6. *Dekker J, Eppink MH, van Zwieten R, de Rijk T, Remacha AF, Law LK, et al. Seven new mutations in the nicotinamide adenine dinucleotide reduced-cytochrome b(5) reductase gene leading to methemoglobinemia type I. Blood. 2001;97:1106-14.*
7. *Nicolas-Jilwan M. Recessive congenital methemoglobinemia type II: Hypoplastic basal ganglia in two siblings with a novel mutation of the cytochrome b5 reductase gene. Neuroradiol J. 2019;32:143-7.*
8. *Gupta V, Kulkarni A, Warang P, Devendra R, Chiddarwar A, Kedar P. Mutation update: Variants of the CYB5R3 gene in recessive congenital methemoglobinemia. Hum Mutat. 2020;41:737-48.*
9. *Cortazzo JA, Lichtman AD. Methemoglobinemia: a review and recommendations for management. J Cardiothorac Vasc Anesth. 2014;28:1043-7.*
10. *Iolascon A, Andolfo I, Russo R, Barcellini W, Fermo E, Toldi G, et al. Summary of Joint European Hematology Association (EHA) and EuroBloodNet Recommendations on Diagnosis and Treatment of Methemoglobinemia. Hemasphere. 2021;5:e660.*
11. *Rehman A, Shehadeh M, Khirfan D, Jones A. Severe acute haemolytic anaemia associated with severe methaemoglobinaemia in a G6PD-deficient man. BMJ Case Rep. 2018;2018.*
12. *Harvey JW, Keitt AS. Studies of the efficacy and potential hazards of methylene blue therapy in aniline-induced methaemoglobinaemia. Br J Haematol. 1983;54:29-41.*
13. *Rosen PJ, Johnson C, McGehee WG, Beutler E. Failure of methylene blue treatment in toxic methemoglobinemia. Association with glucose-6-phosphate dehydrogenase deficiency. Ann Intern Med. 1971;75:83-6.*

14. *Wright RO, Magnani B, Shannon MW, Woolf AD. N-acetylcysteine reduces methemoglobin in vitro. Ann Emerg Med. 1996;28:499-503.*
15. *Wright RO, Woolf AD, Shannon MW, Magnani B. N-acetylcysteine reduces methemoglobin in an in-vitro model of glucose-6-phosphate dehydrogenase deficiency. Acad Emerg Med. 1998;5:225-9.*
16. *Higasa K, Manabe JI, Yubisui T, Sumimoto H, Pung-Amritt P, Tanphaichitr VS, et al. Molecular basis of hereditary methaemoglobinaemia, types I and II: two novel mutations in the NADH-cytochrome b5 reductase gene. Br J Haematol. 1998;103:922-30.*
17. *Rangan A, Savedra ME, Dergam-Larson C, Swanson KC, Szuberski J, Go RS, et al. Interpreting sulfhemoglobin and methemoglobin in patients with cyanosis: An overview of patients with M-hemoglobin variants. Int J Lab Hematol. 2021;43:837-44.*