Case Report

Hereditary Methemoglobinemia Due to Cytochrome b5 Reductase Deficiency

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Abstract : *We report a boy with chronic central cyanosis since birth. He was otherwise asymptomatic with chocolate brown color of blood. Methemoglobinemia was suspected after exclusion of the cardiac and pulmonary diseases. Hereditary methemoglobinemia was considered by his clinical course. The simple bedside procedure could be performed to determine methemoglobinemia by vigorous shaking his blood with oxygen and observing the same chocolate brown in color. Spectrophotometer could be used to confirm this condition by measuring the change in optical density at 630 nm. Cytochrome b5 reductase deficiency was diagnosed by using a simple spot screening test of the enzymatic activity. The patient was treated by the administration of ascorbic acid with reducing of cyanosis and methemoglobin level. Although hereditary enzymopenic methemoglobinemia is a rare autosomal recessive disorder. This disease should be considered in the patient with chronic asymptomatic cyanosis even no history of consanguinity.*

Key Words : ● *Hereditary methemoglobinemia* ● *Cytochrome b5 reductase deficiency* ● *Central cyanosis*

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Methemoglobinemia is not an uncommon condition. It may occur as hereditary or acquired form. Majority of the patients are acquired methemoglobinemia which is caused by acute toxic effects of oxidative stress due to exposure to some specific pharmaceutical or chemical agents such as dapsone, sulfonamides, nitrites, aniline derivatives etc[13]. However, hereditary methemoglobinemia is quite rare. The patients usually present with central cy-

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anosis since birth[4]. Here we report a patient who had central cyanosis since birth and was diagnosed as hereditary methemoglobinemia.

Case report

A 7-year-old boy was admitted to Siriraj hospital due to cyanosis. The patient was born full term with 3,300 grams birth weight by caesarian section because of maternal hypertention at Pataloong hospital. He was observed to have central cyanosis and respiratory distress since birth. He received oxygen therapy together with intravenous medications and was admitted in the newborn unit for 10 days. When he was discharged, he had normal respiration but still had mild bluish discoloration. He had normal growth and development, no dyspnea on exertion and no symptom of hypoxic spell. He was an athlete in his school and had normal life's activity. At age 3 years old, he had been worked up at Pataloong hospital for his mild bluish discoloration by chest X-ray and echocardiogram which were normal. Seven months before this admission, he underwent appendectomy for acute appendicitis without complication but his physician noticed his low oxygen saturation and referred him to Siriraj hospital for further work up. His only brother, aged 13 years, is healthy without skin discolorations. His parents are not relatives but lived in the same province (Pataloong). All of them were healthy without cyanosis. He had no history of using well-water or contacting the chemical substances. The physical examination revealed an intelligent boy with generalized cyanosis. He had normal vital signs. His weight was 26 kg (25^{th} - 50^{th} percentile) and his height was 126 cm (75^{th} percentile). Digital clubbing was not observed. Heart and lung were normal. Liver and spleen were not palpable. Nervous system were grossly intact. The patient's intelligent quotient score was 90 which was normal for his age.

The laboratory works up included complete blood count which revealed hemoglobin (Hb) 16.1 g/dL and hematocrit (Hct) 45.2%. White blood cell count, differential count, platelet count and morphology of red blood cells (RBC) were all normal. Urinalysis and blood chemistry were normal. Hb typing[5,6] revealed Hb A of 85.7% and Hb A2 of 2.6%. Glucose -6- phosphate dehydrogenase (G-6-PD) assay was performed[7] and revealed normal level of 165.6 IU/100 mL RBC (control 150±20 IU/100 mL RBC) Chest X-ray, lung scan, electrocardiogram (EKG) and echocardiography which were performed to exclude underlying cardiopulmonary abnormality were all normal. His blood was chocolate brown in color which did not become red upon vigorous shaking with air. His oxygen saturation measured in room air by pulse oximeter was 90%. Arterial blood gas in room air showed pH 7.4, PaO_2 90 mmHg, $PaCO_2$ 40 mmHg, HCO_3 25.8 mmHg and O_2 saturation 96.8%. His cyanosis was unresponsive to oxygen administration. Under oxygen mask with bag 15 L/min, his arterial blood gas showed PaO_2 189 mmHg and oxygen saturation 99.2%. His blood

was measured the change in optical density at 630 nm by using a spectrophotometer, indicating presence of methemoglobin which was calculated to have 12.6% of total Hb[8,9]. Blood sample was also sent for sulfhemoglobin which was 0%[8,9]. Methemoglobin was undetectable in the blood samples from both parents and brother. A qualitative enzymatic assay of the activity of the cytochrome b5 reductase was performed by measuring the rate of defluorescence of reduced NAD (NADH) in a reduction reaction of dichlorophenol-indophenol (DCIP) in which cytochrome b5 reductase is necessary[10]. The control specimen was defluorescent around 30 minutes while the patient's specimen was not defluorescent until 75 minutes. Prolongation of the defluorescence suggested deficiency of the cytochrome b5 reductase system. Hereditary methemoglobinemia due to cytochrome b5 reductase deficiency was diagnosed. The patient was treated with vitamin C orally 500 mg/day. The medical advices were given to avoid the oxidants that might induce methemoglobin formation. At 2 months' follow-up, the cyanosis reduced clinically with reduction of methemoglobin peak at aforementioned absorption spectrum to 3% and his Hb/Hct also slightly reduced to 14.2 g/dL/ 42.6%. He had normal life style and studied with a good grade in his school.

Discussion

This patient came to medical attention because of central cyanosis since birth. Methemoglobinemia was suspected after exclusion of cardiac and pulmonary diseases. The presence of normal arterial oxygen tensions (PaO_2) and oxygen saturation in arterial blood gas but low oxygen saturation by pulse oximetry in this asymptomatic cyanotic patient is highly suggestive of methemoglobinemia. Acquired methemoglobinemia was ruled out by his history and clinical course. Hereditary methemoglobinemia was considered in this patient. The simple bedside test to determine methemoglobin was done by exposure of the patient's blood which was chocolate brown in color to air or oxygen and it did not change to red color. This finding suggested methemoglobinemia which was confirmed by presence of Hb concentration at absorption spectrum of 630 nm from spectrophotometer[4,8]

Methemoglobin is a form of hemoglobin in which heme iron (ferrous state, Fe^{2+}) has been oxidized to the ferric state (Fe^{3+}). It produces a functional anemia from reducing the oxygen-carrying capacity. In addition, ferric heme groups increase the oxygen affinity of the remaining ferrous heme groups on the same hemoglobin tetramer because the conformation of hemoglobin is changed. So methemoglobinemia shifts the hemoblobin dissociation curve to the left. In this way, methemogloblinemia exerts a dual affect in impairing the supply of oxygen to tissue[2]. Under physiologic conditions, a small amount of oxyhemoglobin slowly auto-oxidizes to methemoglobin which will be reduced to hemoglobin by two enzyme systems.

Diaphorase I also known as nicotinamide adenine dinuclotide (NADH)- dependent methemoglobin reductase or cytochrome b5 reductase, normally accounts for 95% of the reducing capacity of the red blood cells. This linked system also consisting of two electron carriers, cytochrome b5 and the reduced form of NAD. Diaphorase II or nicotinamide adenine dinucleotide phosphate (NADPH) - dependent methemoglobin reductase, supplies the other 5%. Diaphorase II is depend on the hexose - monophosphate shunt as its source of NADPH and can pharmacologically be activated by an exogenous cofactor such as methylene blue to 4 - 5 times of its normal activity; therefore, individuals who are deficient of glucose - 6 - phosphate dehydrogenase (G-6-PD) activity which is important in hexose - monophosphate shunt are not candidates for methylene blue therapy. Administration of methylene blue to such persons may induce hemolysis or methemoglobin formation[1,3]. Normal red blood cells contain less than 1% of methemoglobin. In the absence of an efficient enzymatic reducing system, it is estimated that methemoglobin accumulates at the rate of 2% to 3% per day. A concentration of at least 1.5 g/dL of methemoglobin (approximately 10-15% methemoglobin concentration) in the blood can produce recognizable cyanosis which is resulted from the altered visible spectrum of the abnormal pigments[1,3]. Organs with high oxygen demands, such as cardiovascular system and central nervous system are the first systems to manifest toxicity. Severity of the symptoms depend on the level of methemoglobin and the duration of methemoglobin exposure[1].

Congenital methemoglobinemia is due either to one of the M hemoglobin or to impairment of the enzymatic reduction of methemoglobin. M hemoglobin is the form of abnormal Hb which is held in the methemoglobin state and is inherited according to an autosomal dominant pattern. In this patient, M hemoglobin was ruled out by the history and Hb electrophoresis which revealed no abnormal Hb M bands. Deficiency of cytochrome b5 reductase is relatively common. It is transmitted by autosomal recessive mode of inheritance. The majority of patients have no significant symptom even with deep cyanosis[1,2]. Type I individuals have a deficiency of only the soluble cytoplasmic enzyme. Thus, enzyme activity in tissues other than red cells is normal[1]. Type II disease is the form of the disorder in which the deficiency also exists in nonerythroid cells, such as fibroblasts and lymphocytes. The latter patients are afflicted with a progressive encephalopathy and mental retardation[2,11,12]. The neurologic disorder is probably related to impaired production of unsaturated fatty acids, which play an important role in myelination[1,2]. Several families have been reported to have cytochrome b5 reductase deficiency in red cells, white cells, and platelets but not in other tissues. These affected individuals were designated as having type III deficiency which have no neurologic manifestation as patients in type I deficiency[1,11].

Though this patient had no history of consanguinity in the family, his history of chronic cyanosis and benign clinical course suggested hereditary methemoglobinemia. Cytochrome b5 reductase deficiency was confirmed by qualitative enzymatic assay[10]. Type II deficiency was ruled out due to normal neurologic manifestations, normal development and intelligence. NADH - ferricyanide reductase assay in both RBC and WBC[4,13] will be useful for differentiation of type I and type III deficiency.

In methemoglobinemia patient, normal PaO_2 concentrations are usually found in blood gas analysis. A blood gas machine calculates oxygen saturation from the partial pressure of oxygen and pH levels in the blood[14,15], thus the oxygen saturations from a blood gas machine are usually normal in methemoglobinemia. Pulse oxymetry measures the transmission of two wavelengths of light that was most absorbed by oxyhemoglobin and deoxyhemoglobin. So methemoglobinemia patients can have normal or low arterial blood oxygen saturation by pulse oximetry[16]. Therefore, the values of oxygen saturation obtained by pulse oximetry and a blood gas machine are unreliable in the presence of methemoglobin. The oxygen saturation in this patient measured by pulse oximetry was 90% but measured by a blood gas machine was 96.8% with PaO_2 90 mmHg which were different. Clinicians should be aware of the unreliable of oxygen saturation determined by pulse oximetry and a blood gas machine when an abnormal hemoglobin is present[14].

For the management, conversion of methemoglobin to hemoglobin can be achieved by the administration of methylene blue 1.5-5 mg/kg/day, ascorbic acid (vitamin C) 5-8 mg/kg/day[13] or riboflavin[1,17]. Of these three agents, methylene blue is the most effective drug[1]. Since G-6-PD activity was normal in this patient, methylene blue could be used for the treatment. However, this patient is asymptomatic cyanosis and the level of methemoglobin is not high (12.6% of total Hb). The medication was only given for the cosmetic reason. Thus vitamin C was chosen because it had no side effect and was convenient to administer. The response was observed at 2 months' follow up by reducing clinical cyanosis and methemoglobin level. Mild hypoxic response was also observed in this case with compensated rising of Hb levels (16.6 g/dL). After vitamin C was given for 2 months, the Hb concentration was decreased to 14.2 g/dL. Besides the medical treatment, advice has to be given to the families to be aware of the risk after exposure to some oxidants (drugs or chemical substances) that might induce methemoglobinemia even in persons who are only the carriers of this disorder. Genetic counselling about the course of disease and its mode of transmission should be given to the patients and their families because the risk of having affected offspring is increased in the consanguinous marriage[1,3,4].

Conclusion

Although hereditary enzymopenic methemo-

globinemia is a rare autosomal recessive disorder, this disease should be considered in the patient with chronic asymptomatic cyanosis even no history of consanguinity.

References

1. *Bunn HF. Human hemoglobins : normal and abnormal. In : Nathan DG, Orkin SH, eds. Nathan and Oski's Hematology of infancy and childhood. 5th ed. Philadelphia : W.B. Sainders company, 1998:729-61.*
2. *Beutler E. Methemoglobinemia and other causes of cyanosis. In : Beutler E, Lichtman MA, Coller BS, Kipps TJ, eds. Williams Hematology. 5th ed. New York: McGraw-Hill, Inc., 1995:654-63.*
3. *วรวรรณ ตันไพจิตร. Methemoglobinemia. ใน : มนตรี ตู้จินดา, วินัย สุวัตถี, อรุณ วงษ์จิราษฎร์, ประอร ชวลิตธำรง, พิภพ จิรภิญโญ, บรรณาธิการ. ตำรากุมารเวชศาสตร์เล่ม 2. กรุงเทพมหานคร : โรงพิมพ์เรือนแก้วการพิมพ์, 2541:1484-90.*
4. *Tanphaichitr VS, Mahasandana C, Pung-ammritt P, et al. Type I and II hereditary methemoglobinemia due to NADH - cytochrome b5 reductase deficiency in Thai patients. Int Med 1997;13:81-5.*
5. *Smithies O. An improved procedure for starch gel electrophoresis : further variation in serum proteins of normal individuals. Biochem J 1959;71:585-7.*
6. *Marengo-Row AJ. Rapid electrophoresis and quantitation of hemoglobin on cellulose acetate. J Clin Pathol 1965;18:790-2.*
7. *WHO. Report of WHO Scientific group. Standardization of procedures for the study of glucose - 6 - phosphate dehydrogenase. WHO Tech Rep Ser 1967;366: 5-53.*
8. *Evelyn KA, Malloy HT. Microdetermination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood. J Biol Chem 1938;126:655-62.*
9. *Drabkin DL, Austin JH. Spectrophotometric studies II, preparation from washed blood cells : nitric oxide hemoglobin and sulfhemoglobin. J Biol chem. 1936;112: 51.*
10. *Kaplan JC, Nicolas AM, Hanzlickova - Leroux A, Beutler E. A simple spot screening test for fast detection of red cell NADH - Diaphorase deficiency. Blood 1970;36: 330-3.*
11. *Tanishima K, Tomoda A, Yoneyama Y, Ohkuwa H. Three types of hereditary methemoglobinemia due to NADH - cytochrome b5 reductase deficiency. Adv Clin Enzymol 1987;5:81.*
12. *Higasa K, Manabe JI, Yubisui T, et al. Molecular basis of hereditary methemoglobinemia, type I and II : two novel mutations in the NADH - cytochrome b5 reductase gene. Br J. Haematol 1998;103:922-30.*
13. *Yubisui T, Takeshita M. Characterization of the purified NADH - cytochrome b5 reductase of human erythrocyte as a FAD - containing enzyme. J Biol Chem 1980;255:2454-6.*
14. *Shotelersuk V, Tosukhowong P, Chotivitayatarakorn P, Pongpunlert W. A thai boy with hereditary enzymopenic methemoglobinemia type II. J Med Assoc Thai 2000;83:1380-6.*
15. *Lister G, Fontan JP. Pediatric critical care. In : Behrman RE, Kliegman R, Arvin AM, eds. Nelson Textbook of Pediatrics. 15th ed. Philadelphia : WB Saunders Company, 1996:239-48.*
16. *Yeh J. Poisonings. In : Gunn VL, Nechyba C, eds. The Harriet Lane Handbook. 16th ed. Philadelphia : Mosby, 2002:19-50.*
17. *Hirano M, Matsuki T, Tanishima K, et al. Congenital methemoglobinemia due to NADH methemoblobin reductase deficiency : successful treatment with oral riboflavin. Brit J Haematol 1981;47:353-9.*

Hereditary Methemoglobinemia จากการขาด Cytochrome b5 Reductase Deficiency

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บทคัดย่อ : *ผู้เขียนรายงานผู้ป่วยเด็กชาย 1 ราย มีอาการเขียวทั้งตัวตั้งแต่เกิดโดยไม่มีอาการผิดปกติอื่นๆ นอกจากเลือดมีสีน้ำตาลช็อกโกแลต ผู้ป่วยได้รับการตรวจหัวใจและปอดพบว่าปกติ จากการดำเนินโรคของผู้ป่วยซึ่งเป็นแบบเรื้อรังทำให้นึกถึงภาวะเมทฮีโมโกลบินสูงในเลือดแต่กำเนิดซึ่งมีสาเหตุจากความผิดปกติทางพันธุกรรม ผู้ป่วยได้รับการตรวจโดยการเจาะเลือดแล้วเขย่าให้เข้ากับออกซิเจนพบว่าไม่เปลี่ยนเป็นสีแดงเหมือนเลือดปรกติทำให้วินิจฉัยเบื้องต้นได้ การตรวจโดยเครื่อง spectrophotometer เพื่อหาปริมาณของ เมทฮีโมโกลบิน โดยดูการเปลี่ยนแปลงความเข้มข้นของแสงที่ 630 นาโนเมตร จะช่วยให้วินิจฉัยได้แน่นอนและ การตรวจหาเอ็นไซม์ cytochrome b5 reductase โดยวิธี spot screening test ทำให้สามารถวินิจฉัยภาวะพร่องเอ็นไซม์ cytochrome b5 reductase ได้ ผู้ป่วยได้รับการรักษาด้วยวิตามินซี หลังการรักษาพบว่าอาการเขียวและระดับเมทฮีโมโกลบินในเลือดลดลง ถึงแม้ว่าภาวะเมทฮีโมโกลบินสูงในเลือดแต่กำเนิดที่เกิดจากการขาดเอ็นไซม์ cytochrome b5 reductase ซึ่งมีการถ่ายทอดทางพันธุกรรมแบบยีนด้อยจะพบได้น้อย แต่เมื่อพบผู้ป่วยที่มีอาการเขียวแต่กำเนิดโดยที่ไม่มีอาการผิดปกติอื่นๆ ควรนึกถึงโรคนี้ด้วย แม้ว่าจะไม่มีประวัติการแต่งงานในเครือญาติก็ตาม*

Key Words : ● *Hereditary methemoglobinemia* ● *Cytochrome b5 reductase deficiency* ● *Central cyanosis*

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คติธรรมนำกำจัดจุดอ่อน

ยอมด้วยเหตุผล ทนด้วยเมตตา

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