Familial Psychomotor Delay of an Uncommon Cause: Type II Congenital Methemoglobinemia

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ABSTRACT: Methemoglobinemia is due to oxidization of divalent ferro-iron of hemoglobin to ferri-iron of methemoglobin (MetHb) which is incapable of transferring oxygen to tissues. This disease may be acquired by intoxication with oxidizing agents or inherited with a mutation of CYB5R3, the gene coding for the methemoglobin reductase or cytochrome B5 reductase 3 responsible for the reduction of MetHb to hemoglobin. We report the case of 2 sisters aged respectively of 15 and 8 months. They were born to a second-degree consanguineous marriage with a history of precocious and unexplained deaths in 3 relatives. Both sisters presented neurological features including psychomotor retardation, microcephaly, and axial hypotonia. Cerebral magnetic resonance imaging revealed cerebral atrophy in both cases associated with hypoplasia of the corpus callosum in the younger child. The association of neurological disability, cyanosis, and hypoxemia prompted a search for methemoglobinemia, with MetHB levels respectively of 26% and 15.8%in the 2 sisters. Initial treatment was based on methylene blue, then ascorbic acid. The genetic study revealed a c.463+8G>C mutation of CYB5R3 confirming the diagnosis of methemoglobinemia type II. The diagnosis of methemoglobinemia, although rare, should be considered in the presence of psychomotor retardation with cyanosis and subacute onset hypoxemia, especially in the presence of a family history.

KEYWORDS: Methemoglobinemia, cyanosis, cytochrome b5 reductase, CYB5R3 gene, brain atrophy, psychomotor delay

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Introduction

Oxygen transport by hemoglobin requires the presence of bivalent iron. Oxidation of divalent ferro-iron to ferri-iron induces methemoglobin, which is incapable of transporting oxygen.[1,2] Under physiological conditions, 0.5% to 2% of hemoglobin is in the form of methemoglobin. When the proportion of methemoglobin exceeds 10% to 20%, symptoms such as cyanosis, dizziness, and confusion may occur.[3]

Cychrome B5 reductase 3 encoded by the CYB5R3 gene is an enzyme that catalyzes the reduction of methemoglobin to hemoglobin, keeping methemoglobin levels below 2%. The appearance of high methemoglobin concentrations may be linked to a cytochrome B5 reductase 3 deficiency.[3,4] Methemoglobinemia may be acquired or congenital. The congenital type is classified as type I, II, or IV according to the presence of other associated clinical features. Acquired methemoglobinemia is usually induced by oxidizing agents such as drugs (eg, dapsone, sulfamethoxazole, and nitrate).[5-7]

Less than 60 patients with recessive hereditary methemoglobinemia were described from the mid-1950s to date. In contrast with detailed biochemical or genetic studies, the clinical features and long-term outcomes of patients are poorly documented.[8] The neurological phenotype is consistent with a severe neurodevelopmental disorder characterized by global developmental delay; congenital or acquired microcephaly; tetraparesis; spasticity; movement disorders (choreoathetosis and dystonia), and drug-resistant epilepsy in a smaller percentage of patients (16%). Life expectancy usually does not exceed 10 years. Survival appears to depend on 2 factors: swallowing function, determining the risk of respiratory infections and failure to thrive; and medical, paramedical, and familial management.[8,9]

Cases Presentation

Case 1

The first patient was aged 15 months and was born to a second-degree consanguineous marriage. She was born full-term. Pregnancy and delivery were uncomplicated. Birth measurements were normal.

The family history revealed, on the mother's side, a precocious death of 1 sister and 2 brothers. They had a psychomotor delay, axial hypotonia, and failure to thrive. Death occurred between the ages of 6 months and 5 years by severe bronchopulmonary infection. No etiological diagnosis was retained for these 3 cases.

Our patient was admitted for the first time in the pediatrics department for psychomotor delay evolving since the age of 5 months. On examination, the weight was 6.9 kg (−3SD, according to the WHO growth chart), the height was 73 cm (−2SD, according to the WHO growth chart), and the head circumference was 41 cm (−3SD, according to the WHO growth chart). She could not support her head and had severe swallowing disturbances. She did not turn to the sound, did not watch the surroundings, or smile. A spastic tetraparesis was associated with the axial hypotonia.

No cyanosis was observed and oxygen saturation was not measured. Investigations including a karyotype, a thyroid function, and a chromatographic analysis of amino acids, and organic acids on plasma and urine respectively by ion exchange chromatography and gas chromatography/mass spectrometry were unremarkable.

Cerebral brain magnetic resonance imaging (MRI) showed a quadriventricular dilatation without signs of cerebrospinal

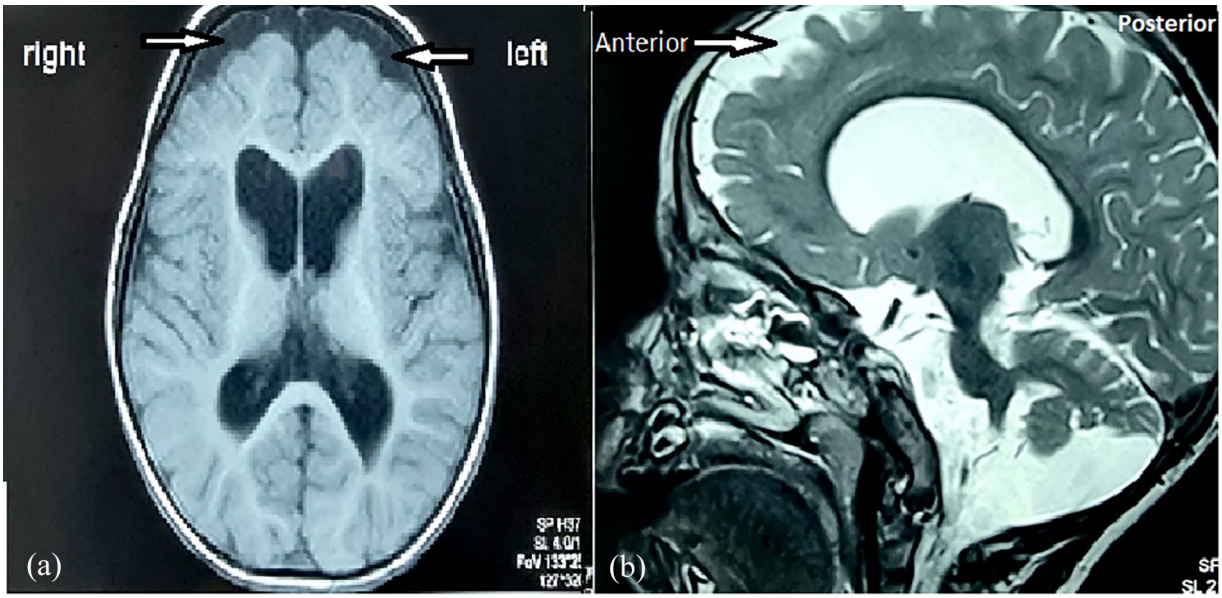

Figure 1. Magnetic resonance imaging: (a) axial T1 and (b) sagittal T2: quadriventricular dilatation without signs of cerebrospinal resorption and bilateral frontal cortical atrophy (horizontal arrows).

Figure 2. Blood sample colored dark brown from patient 1.

resorption and associated with a bilateral frontal cortical atrophy (Figure 1).

Chest X-ray and cardiac ultrasound were unremarkable.

Six months later, she was readmitted, for facial cyanosis. The oxygen saturation level was at 92%. A methemoglobin assay was performed, and the blood sample was dark brown (Figure 2), with a level of MetHb equal to 26%.

There was no history of oxidant medication intake before this episode.

The patient received methylene blue (1 mg/kg) and ascorbic acid (500 mg/day) with a control methemoglobin level of 1.6% after 2 days.

The molecular analysis of the CYB5R3 gene was performed to confirm the hereditary origin.

For the genetic study, 3 to 5 mL blood samples were collected from the proband and her parents. DNA was extracted from the blood using a commercially available DNA extraction kit (QIAGEN, Germantown, MD, USA) following standard protocols. Chromosomal Microarray Analysis was performed using Infinium CytoSNP-850 K BeadChip (SNP-array, Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Array scanning data were generated on the Illumina iScan system, and the results were analyzed by Bluefuse Multi 4.4 software. The confirmation and segregation tests were performed on the patient's and her parents' DNA by conducting real-time PCR on CYB5R3 using a SYBR Green assay.[10]

Genetic analysis of CYB5R3 (NM_000398) detected a homozygous mutation in intron 5: c.463+8G>C in favor of the diagnosis of congenital methemoglobinemia.

DNA change (genomic) relative to Genome Reference Consortium (hg38/ GRCh38) was g.42628144C>G.

This mutation was described as pathogenic by Vieira (1995), Blood 85, 2254.[11] It was inherited from heterozygous carrier parents

In front of the early onset of psychomotor delay, cyanotic hypoxemia, MetHb level >20%, and the result of the genetic test, the diagnosis of type II methemoglobinemia was retained.

Genetic counseling was provided to the parents.

Case 2

Once the diagnosis was made in the first patient, a clinical evaluation was proposed for her sister, aged 8 months. She was born full-term. Pregnancy and delivery were uncomplicated. On examination, she had an axial hypotonia, a poor ocular pursuit, and a mild perioral cyanosis.

Her methemoglobin level was at 15.8%. She was put on methylene blue and thereafter on Vitamin C with a control level of 0% after 1 day.

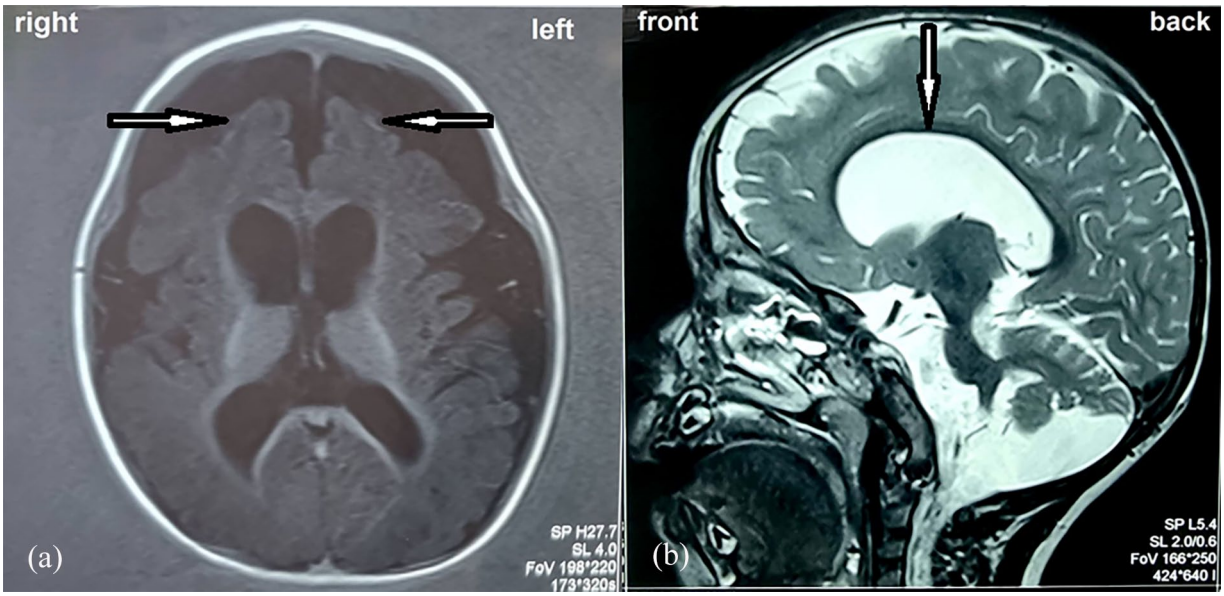

Figure 3. Magnetic resonance imaging: (a) axial T1, (b) sagittal T2: diffuse enlargement of subarachnoid spaces, cortico-subcortical atrophy (horizontal arrows) and hypogenesis of the corpus callosum (vertical arrow).

Her brain magnetic resonance imaging (MRI) showed cerebral atrophy with hypogenesis of the corpus callosum (Figure 3).

Genetic sequencing of CYB5R3 has detected the same homozygous mutation as her sister **c.463+8G>C** in favor of the diagnosis of congenital methemoglobinemia.

The diagnosis of methemoglobinemia type II within the 2 sisters explained the death of the mother's siblings who presented with the same clinical features as in our 2 patients.

Discussion

The cases we reported highlighted a rare case of psychomotor delay. The pediatrician must think about this uncommon etiology and search for its signs such as perioral cyanosis and low oxygen level especially when cardiac and respiratory disorders have been ruled out.

The dark brown color of blood samples must also draw attention to the diagnosis of methemoglobinemia.[3]

Methemoglobinemia is due to a lack of reduction of methemoglobin to hemoglobin that is catalyzed by the enzyme cytochrome B 5 reductase 3, known as methemoglobin reductase.[8,9] There are 2 main systems: NADH-dependent methemoglobin reductase type I or diaphorase, which is the main system for converting methemoglobin to hemoglobin and ensures the stability of its levels in the body. The other is NADPH-dependent methemoglobin II, which does not play a physiological role but becomes functional in the presence of oxidizable compounds such as methylene blue, demonstrating its effectiveness in the treatment of methemoglobinemia.[7,12] Methemoglobin can also be reduced by ascorbic acid and glutathione, which is why both of our patients were treated with methylene blue during the acute phase and ascorbic acid as background therapy.[13]

Methemoglobinemia can be acquired or hereditary. The acquired type is characterized by acute cyanosis in previously healthy patients. The main responsible agents are oxidizing drugs such: as dapsone, sulfonamides, titracaine, cloroquine, and benzocaine; various substances used in industry, such as polyphenols, hydrazines, aniline, and nitrobenzene; oxidizing agents likely to be brought in by food (well water, spinach, fertilizers, and preservatives). Some of the agents responsible are not toxic in vitro, but become toxic in vivo through the formation of an oxidizing metabolite: this is the case of nitrates reduced to nitrite by intestinal microbial flora.[4-14]

The congenital type presents in different forms: methemoglobinemia linked to diaphorase enzyme deficiency, methemoglobinemia type I and methemoglobinemia type II, which are autosomal recessive, due to a CYB5R3 gene mutation, and methemoglobinemia type IV, which is also autosomal recessive, linked to a CYB5A gene mutation.[13]

The clinical features of methemoglobinemia depend on the percentage of methemoglobin:

> (a) less than 10%: the patient may be asymptomatic or may present a pale facies, (b) between 10% and 30%: symptoms range from no symptoms to cyanosis (perioral and peri-ungeal), vertigo, and confusion, (c) between 30% and 50%: the color of the blood changes to dark brown, and symptoms are more severe: dyspnea, palpitations, dizziness, headache, cyanosis and confusion, (d) higher than 50% metabolic acidosis and altered neurological status generally occur, with the risk of convulsions and dysarrhythmias, and (e) when the level exceeds 70%, the outcome can be rapidly fatal.[3]

Associated clinical features can suggest the type of methemoglobinemia. In our patients, neurological damage was associated. A review of the literature on type II methemoglobinemia shows that the onset of symptomatology ranges from birth to the first year of life, with an average age of

discovery of 5 months as in the second reported case.[3,4] The main symptoms are cyanosis with delayed development. Microcephaly, strabismus, and pyramidal signs may be seen, as well as extrapyramidal movement disorders (dystonia and choreoathetosic movements). Epilepsy may be observed and is always drug-resistant.[8,15]

Hypotonia with psychomotor delay was the main feature in our patients. The family history with a fatal outcome at an early age was highly suggestive of the inherited pattern of the disease.

Regarding brain imaging, the main abnormalities are brain atrophy, cerebellar atrophy, basal ganglia atrophy/hypoplasia, and white matter anomalies.[8,16] In our cases, an MRI was performed as a routine assessment for psychomotor retardation showing brain atrophy.

Genetic study confirms the diagnosis of congenital methemoglobinemia, which is usually associated with a mutation in the CYB5R3 gene.[17] Several mutations have been reported and have provided great information on the clinical and biochemical differences between type I and type II. Type I is more often associated with missense mutations that result in amino acid substitution responsible for enzymatically active but unstable proteins correlating with the benign clinical course of the disease. The type II is usually due to full stops or deletions located in consensus Flavin Adenine Dinucleotide or NADH-binding sites of the enzyme, causing loss of protein expression or enzymatically inactive proteins leading thus to severe neurological impairment.[1,5,14]

In our 2 patients, a mutation type **c.463+8G>C** in the fifth intron was identified. This mutation was described for the first time in 1995 by Vieira et al. It is responsible for the abnormal splicing of primary transcripts, resulting in a frameshift with a premature STOP codon. This mutation is classified as pathogenic.[11]

Hirono et al, reported that cytb5 reductase deficiency is involved in the desaturation of fatty acids. Thereafter, the reduced production of unsaturated fatty acids possibly leads to central dysmyelination and encephalopathy.[18]

While cyanosis can be treated by methylene blue, riboflavin, or ascorbic acid, there is currently no way of preventing or treating the neurological deterioration associated with type II Methemoglobinemia.[18,19]

The methylene blue dose is 1 to 2 mg/kg intravenously over 5 minutes. The dose can be repeated in 30 to 60 minutes if significant symptoms or levels remain above the treatment threshold. Although methylene blue administration is controversial in the setting of G6PD deficiency, it is not contraindicated and should be administered cautiously and judiciously. Doses of methylene blue observed to produce hemolysis in patients with G6PD deficiency have been observed to be over 5 mg/kg, which is more than twice the recommended dose in methemoglobinemia.[19]

When treatment with methylene blue is ineffective or not recommended, additional options may include ascorbic acid (0.2-1.0 g/day orally in divided doses), riboflavin (20-60 mg/day), exchange transfusion, or hyperbaric oxygen therapy.[3]

Our 2 sisters had regular follow-ups. The oldest died at the age of 5 years from hypoxic pneumonia. The other had a stabilized condition under vitamin C and neurophysical rehabilitation

Conclusions

Methemoglobinemia is a rare condition that may be acquired or hereditary.

Type II methemoglobinemia presents a more specific form, with neurological damage manifested by severe psychomotor retardation, hypotonia, microcephaly, and neuroimaging abnormalities, essentially cerebral atrophy. The diagnosis of methemoglobinemia, although rare, should be considered in the presence of psychomotor retardation with cyanosis and subacute onset hypoxemia, especially in the presence of family history or consanguinity among the parents. Genetic testing is essential to identify the mutation and provide genetic counseling to parents.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The guardians gave written informed consent for publication

Author contributions

Hager Barakizou: Conceptualization and Writing—review & editing; Selma Chaieb: Data curation, Formal analysis, and Writing—original draft.

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