Methylmalonic Acidemia in two Filipino Children

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ABSTRACT

We describe two Filipino infants with methylmalonic acidemia, an autosomal recessive organic acid disorder with severe metabolic acidosis as a prominent clinical feature. Clinical course, diagnosis and management of these patients are discussed.

Key Words: methylmalonic acidemia, metabolic acidosis, autosomal recessive

Introduction

Methylmalonic acidemia (MMA) is an inborn error of organic acid metabolism caused by a defect in the conversion of methylmalonyl CoA to succinyl CoA. The reaction is catalyzed by the enzyme methylmalonyl CoA mutase (MCM) which requires adenosylcobalamin (Ado-Cbl) as a co-factor (Figure 1).

Mutations involving the MCM apoenzyme are designated mut^0 if there is no detectable enzyme activity or mut^- if residual activity can be detected under certain conditions. At least 5 different defects in the intracellular metabolim of cobalamin have been identified to cause defects in the biosynthesis of the cofactor adenosylcobalamin (Figure 2). These are designated as *cbl A, cbl B, cbl C, cbl D* and *cbl F.* Defects in *cbl A* and *cbl B* cause MMA only, and present clinically as indistinguishable from mutase defects. In patients *with cbl C, cbl D* and *cbl F* defects, synthesis of both adenosylcobalamin and methylcobalamin are impaired causing homocystinuria in addition to MMA. These disorders do not usually present with metabolic acidosis.[1]

Herein, we report 2 Filipino children with MMA who both presented early in infancy with hypotonia, severe metabolic acidosis, ketonuria, hyperammonemia and pancytopenia.

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Clinical Reports

Patient 1

The male proband was the third child of non-consanguineous parents. He was born full term to a 27 year old gravida 6 para 3 (2022) mother after a pregnancy complicated by preterm labor at 7 months gestation. The first two pregnancies resulted in spontaneous abortions at 5-6 months age gestation. The second liveborn child died at 10 days of life of an undiagnosed illness with symptoms of irritability, vomiting and hypotonia (Figure 3).

The proband was delivered by cesarean section due to breech presentation. His birth weight was 2.5 kg which was small for gestational age. At birth, he had poor cry, poor activity and hypotonia. He had bouts of vomiting on the 48th hour of life. Initial blood investigations showed leukopenia, thrombocytopenia and uncompensated metabolic acidosis. He was treated as a case of neonatal sepsis, but despite aggressive antibiotic therapy, no improvement was noted.

Urine metabolic screening done on the 18th day of life revealed an increased level of methylmalonic acid. Urinary organic acid profile by gas chromatography-mass spectrometry (GC-MS) showed gross increase in methylmalonate and methylcitrate. Serum vitamin B12 level was normal. Serum ammonia was 52 umol/L which was normal for age.

He was then put on a restricted protein diet and was started on a special milk formula (XMTVI analog SHS) devoid of methylmalonic acid precursors. Vitamin B12 and carnitine were started. He then showed gradual improvement in his general well being accompanied by resolution of metabolic acidosis, and was discharged improved on the 40th day of life.

His development was at par with his chronological age. At the time of examination around 2 months old, he was alert, active and with good tone. His head circumference was 40 cm (50th percentile), weight was 2.7 kg (<3rd percentile), and length was 48 cm (<3rd percentile). Physical and neurologic examinations were normal.

He never had seizures and managed to thrive well during the first few months of life. Serial evaluation of methylmalonic acid levels in urine showed lack of significant

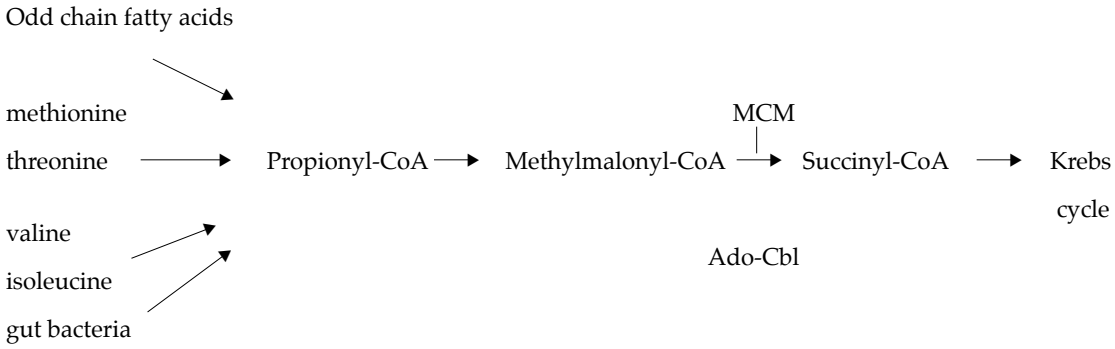

Figure 1. Schematic diagram of pathway

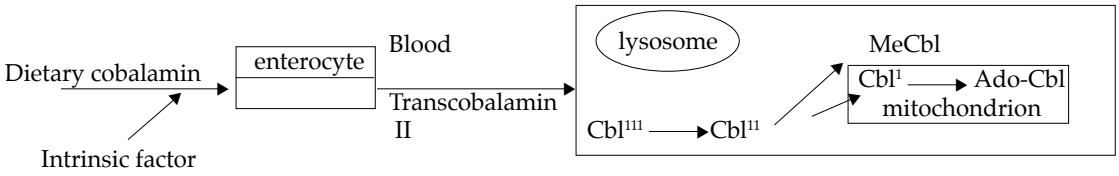

Figure 2. Metabolism of vitamin B12: Dietary cobalamin B12 is bound to gastric instrinsic factor. It is absorbed in the ileum and transported in the blood bound to transcobalamin II (TC II). It is then taken up by the cell, released from TC II in the lysosomes, and oxidized to Cbl^{III} in the cytosol. This compound is further processed either to cytosolic cobalamin (MeCbl) or to mitochondrial adenosylcobalamin (Ado-Cbl).

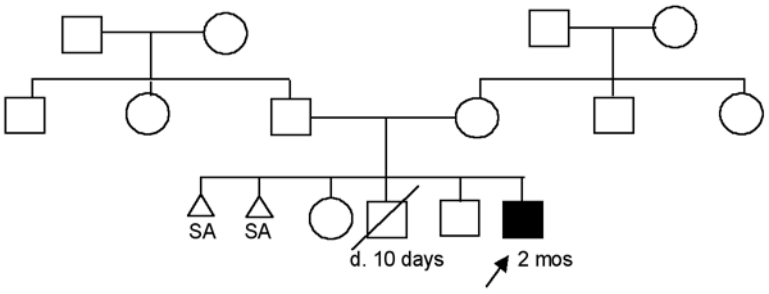

Figure 3. Pedigree of Patient 1

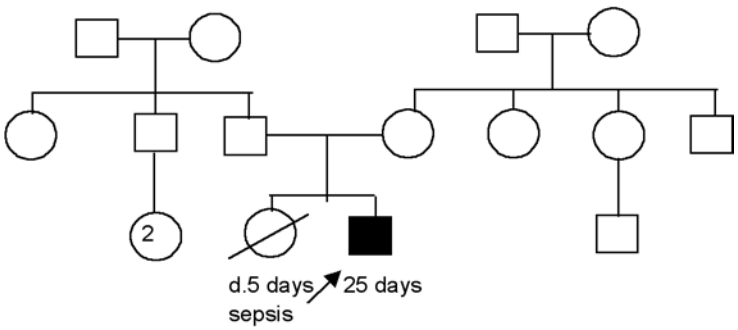

Figure 4. Pedigree of Patient 2

reduction in urine MMA even with pharmacologic doses of oral vitamin B12 at 1 mg per day.

At 2½ and 3½ months of age, he had bouts of diarrhea but these were not associated with any metabolic decompensation and he was able to recover and gain weight in between episodes.

At 5 months of age, he was admitted for a severe bout of pneumonia accompanied by intractable metabolic acidosis. He rapidly deteriorated with accompanying signs of compromised cardiac and renal functions. He died 48 hours later.

Patient 2

This male proband was the second child of a non-consanguineous couple whose first born child died at 6 weeks old because of severe metabolic acidosis that was not comprehensively investigated. (Figure 4).

He was born full term to a 29 year old gravida 2 para 1 mother after a pregnancy complicated by urinary tract infection at 3 months gestation. He was delivered via cesarean section due to maternal pre-eclampsia. He had good Apgar scores and birth weight was 2.59 kg which was small for gestational age. He had jaundice within the first 24 hours of life and was treated as a case of neonatal sepsis. He was discharged improved on the 5th day of life.

He was apparently well until the 11th day of life when he had episodes of vomiting which was later accompanied by poor suck, poor cry and tachypnea. He was then admitted with a primary consideration of sepsis neonatorum. Laboratory investigations showed severe metabolic acidosis with a high anion gap despite aggressive correction, hyperammonemia, ketonuria, normocytic anemia and leukopenia. Urine metabolic screening showed methylmalonic aciduria with elevated lysine and glycine. Urine organic acid analysis showed increased levels of methylmalonate and methylcitrate consistent with methylmalonic aciduria. He was then referred to our institution for further evaluation and management.

At the time of examination on the 25th day of life, his head circumference was 37 cm (50th percentile), weight was 2.2 kg (<3rd percentile), and length was 49 cm (25th percentile). He was rousable, pale and was in respiratory distress. Pertinent physical findings showed hepatosplenomegaly, bilateral hydrocoeles, generalized hypotonia with intact primitive reflexes.

Repeat investigations showed severe anemia with marked hypochromia and macrocytosis, metabolic alkalosis and normal serum ammonia. Chest X-ray and 2D echocardiography showed a dextroversed heart with no intracardiac structural abnormalities. Serum vitamin B12 was normal. Baseline renal function was likewise normal. Blood cultures had growth of *Achromobacter* and *Alcaligenes* species.

He eventually improved with protein restriction, special milk formula, caloric supplementation and vitamin B12 and carnitine administration.

However, 2 weeks after discharge, he was again admitted for infectious diarrhea that was complicated by severe metabolic acidosis and sepsis. He recovered fully after 10 days and was sent home improved. At 3 months of age, his anemia was very prominent with a hemoglobin of 66 g/L. The peripheral blood smear was compatible with megaloblastic anemia with signs of iron deficiency. Intramuscular injections of hydroxocobalamin were started. Transfusion with packed red cells was also given. At 4 month of age, he had an episode of generalized tonic-clonic seizure. Cranial CT scan revealed cortical atrophy with corpus callosum dysgenesis. He was then maintained on anticonvulsant with resultant adequate control of seizures. The iron deficiency anemia though was persistent for which he was given ferrous sulfate. Serial evaluation of urine methylmalonic acid by high voltage electrophoresis on the first, second and third week after B12 administration showed no significant differences in the intensity of methylmalonic acid in the urine.

A formal developmental assessment done at 4 months of age showed global developmental delay. He was then started on physical therapy. Although he showed good developmental progress and growth, he remained developmentally delayed. He was maintained on daily oral supplementation of vitamin B12, carnitine, folic acid and low protein diet. He was maintained on Phenobarbital and seizures were controlled since 4 months of age. His clinical course was complicated by frequent respiratory tract and gastrointestinal infections, some of which were associated with metabolic decompensations that were easily managed. At 3 years of age, he was admitted for pneumonia and eventually died of severe metabolic acidosis and sepsis after 72 hours.

Discussion

Organic acidemias are disorders of intermediary metabolism that lead to accumulation of organic acids in biologic fluids, disturbance of acid-base balance, and derangement of intracellular biochemical pathways.[2]

Methylmalonic acidemia is one of the most common among these disorders and may affect as many as 1: 29,000 newborns[3]. It is associated with clinical phenotypes ranging from fulminant neonatal acidosis and death to benign asymptomatic methylmalonic aciduria.[3,4] Genetic defects causing MMA are classically categorized by somatic cell complementation studies as either cobalamin (*cbl*) defects due to mutations in genes required for provision of the adenosylcobalamin cofactor, or *mut* defects due to mutations in the gene encoding the MCM apoenzyme.[5]

As with other organic acidemias, MMA presents with a variety of signs and symptoms. Single symptoms are not characteristic or diagnostic, thus clinical awareness coupled with appropriate laboratory investigation is required for prompt diagnosis.[6]

Such was the case of the two patients just described, whose symptomatology mimicked neonatal septicemia at the onset, as they both presented with vomiting, lethargy,

hypotonia and intractable metabolic acidosis. However, with a family history of a sibling who had died of a similar clinical course and the poor response to antibiotic therapy, an inborn error of metabolism was suspected.

Laboratory findings showed normal serum cobalamin levels, metabolic acidosis, ketonuria/ketonemia, hyperammonemia, glycinuria, pancytopenia and elevated urine levels of MMA.

Patient 1 was suspected to have apoenzyme deficiency based on the following: early onset of symptoms with stormy clinical course, normal homocysteine level, apparent non-responsiveness to B12 and early death. Patient 2, because of the early and severe clinical presentation that was also associated with B12 unresponsiveness, was likewise suspected to have apoenzyme mutase deficiency. However, an adenosylcobalamin deficiency (either *cbl A* or *cbl B*) could not be entirely ruled out. Defects of both adenosylcobalamin and methylcobalamin synthesis due to abnormal cystosolic and methylcobalamin metabolism of cobalamins *cbl C, D and F* were excluded due to the absence of homocystinuria accompanying the methylmalonic acidemia.

Cobalamin complementation studies and enzyme activity levels on skin fibroblasts were not done due to local unavailability. In the absence of such studies, the diagnosis of MMA was therefore based on the clinical features and biochemical findings of elevated urine methylmalonic acid, with accompanying hyperglycinemia, hyperammonemia and pancytopenia.

The primary block in the conversion of methylmalonyl CoA to succinyl CoA resulting either from defects in the apoenzyme or in the synthesis of the adenosylcobalamin cofactor explains the accumulation of methylmalonate in blood and urine. The accumulation of CoA 'trapped" intracellularly as methymalonyl CoA, could lead to an insufficiency of this widely utilized enzyme, and secondarily, to impaired carbohydrate metabolism and subsequent acidosis. Inhibition of the glycine cleavage enzyme and of carbamoyl phosphate synthase I (CPS I), an enzyme needed by the urea cycle for the metabolism of ammonia, are inhibited by the accumulated organic acids causing hyperglycinemia and hyperammonemia respectively.[1] As a further consideration, methylmalonate inhibits the growth of marrow cells in a concentration dependent fashion leading to pancytopenia.[1] This suggests that patients with MMA are immunocompromised and the decreased resistance to infection can lead to a serious course as seen in our two patients.[6,7]

No consistent pattern of neurological deficit was seen in patients with MMA and seizures were not commonly reported.[8] Instead, most of them present with extrapyramidal symptoms because of the primary involvement of the basal ganglia specifically the globus pallidus which is thought to be especially susceptible to hypoxic or ischemic damage because of marginal circulatory deficiency.[9] The deranged organic acid metabolism in the brain can lead to a compromised neurologic outcome. Proposed mechanisms include "infarctions" due to vasoconstriction brought about by severe acidemia, inhibition by MMA of succinate dehydrogenase which is a key enzyme of Krebs cycle, thereby blocking aerobic glucose oxidation, and induction of other defects in energy metabolism by inhibiting gluconeogenesis and restriction of ketogenesis.[10-11] Hyperammonemia also contribute to brain dysfunction by altering cerebral perfusion pressure and it has deleterious effects on the properties of the blood brain barrier, brain energy metabolism and brain electrophysiology.[12]

Patient 2 had seizure episodes but has never exhibited extrapyramidal system disease. His cranial CT scan finding was limited to cortical atrophy which was most probably due to the neuronal or glial cell injury from the accumulation of organic acid metabolites. The finding of corpus callosum dysgenesis represents a defect in neuronal cell migration and proliferation early in fetal life and may only be incidental. Both the cortical atrophy and corpus callosum dysgenesis could be underlying etiologies for the patient's seizure disorder. Patient 1, on the other hand did not show any significant neurological abnormality and was developmentally at par until 2 months of age. Unfortunately, due to his early death, the neurological and developmental outcome were not ascertained.

Two treatment regimens for children with methylmalonic acidemia should be used in tandem as soon as a diagnosis of MMA is made i.e. a diet restricted in protein with supplementation of a special formula restricted in amino acid precursors of methylmalonate (excluding methionine, threonine, valine and isoleucine – XMTVI) and supplementary cobalamin (Cbl). Such measures should decrease the circulating concentrations of methylmalonate and propionate.

In a review of Matsui et al on the natural history of inherited methylmalonic acidemias, none of the children designated with mutase *mut* ^{0} *or mut* $^{-}$ genotype responded to cobalamin supplementation. Almost 90% of the *cbl A* patients and only about 40% of *cbl B* patients showed such a response. The fraction (60%) of *cbl* patients unresponsive to cobalamin supplements presumably has such complete adenosyltransferase deficiency that adenosylcobalamin synthesis cannot be augmented by cobalamin supplements, as it apparently can in the *cbl B* patients with leaky mutations that permit *in vivo* responsiveness. Patients in the *cbl A* group were uniformly responsive suggesting either that the responsible mutations are generally leaky allowing mass action to result in more adenosylcobalamin synthesis or that alternate pathways requiring high substrate concentrations exist in cells.[13]

Carnitine supplementation is a useful therapeutic adjunct presumably by replenishing intracellular and extracellular stores of free carnitine that are depleted in affected patients because of exchange with excess methylmalonyl CoA and propionyl CoA. The recognition that gut bacteria may contribute significantly to propionate production has led to the administration of oral metronidazole therapy in these

patients.[1]

The long term outcome in affected patients depends considerably on the nature of the biochemical lesion causing methylmalonic acidemia and on the metabolic control. *Mut*^{0} group has the poorest prognosis while *cbl A* (i.e. the group biochemically most responsive to Cbl supplementation) patients had the best outcome.The *cbl B* and *mut* – groups were intermediate, with about equal fractions in each group being found in the alive and well, the alive and impaired, or the deceased category.[1]

The families of our patients received genetic counselling. Methylmalonic acidemia being an autosomal recessive condition carries a 25% recurrence risk during each pregnancy.

Methylmalonic aciduria is a very serious but treatable condition, and some patients do well if treatment can be started promptly. Increased awareness among clinicians that non specific clinical features such as vomiting, hypotonia, metabolic acidosis and failure to thrive can be presenting signs of an inherited metabolic disorder may lead to a more timely diagnosis as well as reduced morbidity and mortality.

References

1. Fenton WA, Gravel RA, Rosenblatt DS. Disorders of propionate and methylmalonate metabolism. In Scriver CR, Beaudet AI, Sly WS, Valle D, eds.The metabolic and molecular bases of inherited disease by Scriver et al. 8th edition. Mc Graw Hill, 2001; 2165-2193.
2. Ozand PT, Gascon GG. Organic acidurias: A review. Part 1. J Child Neuro. 1991; 6:196-211.
3. Coulombe JT, Shih VE, Levy HL. Massachusettes metabolic disorders screening program II. Methylmalonic aciduria. Pediatrics. 1981; 67:26-31.
4. Treacy E, Clow C, Mamer A, Scriver C. Methylmalonic acidemia with severe biochemical but benign clinical phenotype. J Pediatr 1993; 122: 428-429.
5. Methylmalonic aciduria due to methylmalonyl CoA mutase deficiency. Online Mendelian Inheritance in Man. Available at http://www.ncbi.nlm.nih.gov. Accessed August 5, 2004.
6. Brandt N. Symptoms and signs in organic acidurias. J Inher Metab Dis. 1984; 7: 23-27.
7. Henriquez A, El Din A, Ozand PT, Subramanyan SB, Al Gain SI. Emergency presentations of patients with methylmalonic acidemia, propionic acidemia and branched chain amino aciduria (MSUD). Brain Dev. 1994; 16 : 86-93.
8. Shevell M, Matiaszuk N, Ledley F, Rosenblatt D . Varying neurological phenotypes among *mut*^{0} and *mut* $^-$ patients with methylmalonyl CoA mutase deficiency. Am J Med Genet. 1993; 45:619-624.
9. Heidenreich R, Natowecz M, Hainline B, Berman P, Kelly R, Hillman R, et al. Acute extrapyramidal syndrome in methylmalonic acidemia: "metabolic stroke" involving the globus pallidus . J Pediatr. 1988; 113 : 1022-1027.
10. Wajner M, Coehlo J. Neurologic dysfunction in methylmalonic acidemia is probably related to the inhibitory effect of methylmalonate on brain energy production. J Inher Metab Dis. 1997;l 20: 761-768.
11. Enns G, Barkovich A, Rosenblatt D, Fredrick D, Weiseger K, Ohnstad C, et al. Progressive neurologic deterioration and MRI changes in *cbl C* methylmalonic acidemia treated with hydroxocobalamin. J Inher Metab Dis. 1999; 22: 599-607.
12. Surtees R, Leonard JV. Acute metabolic encephalopathy: a review of causes, mechanisms and treatment. J Inher Metab Dis. 1989;12 : 42-54.
13. Matsui S, Mahoney M, Rosenberg L. The natural history of the inherited methylmalonic acidemias. N Eng J Med. 1983; 308: 857-861.