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Case Report

New-born screening of metabolites with liquid chromatography-mass spectrometry and gas chromatography–mass spectrometry: a metabolic and molecular signatures methyl malonic aciduria with combined oxidative phosphorylation deficiency-10: a rare case report

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ABSTRACT

An uncommon autosomal recessive organic acid disease is malonic aciduria. This disease may be easily identified and included in the NBS programmes by means of the widespread use of tandem mass spectrometry's in the study of the amino acid/acylcarnitine profile using dried blood spots (DBS) for newborn screening. In Tamil Nadu, we reported the first screened and diagnosed with malonic aciduria by newborn screening (NBS) in early neonatal period. The patient possesses a malonyl-CoA decarboxylase genetic variation not previously described. This disease should be distinguished from a related malonic and methylmalonic aciduria problem. Malonic aciduria's clinical phenomenology varies and pathogenesis is not completely known. The proper treatment regimen, nutritional therapy or frequent follow-up to these individuals are not guided or recommended. The majority of current treatment data is based on a single research or case report.

Keywords: Newborn screening, Tandem mass spectrometry, Liquid chromatography-mass spectrometry, Gas-chromatography-mass spectrometry, Clinical exome sequencing

INTRODUCTION

Genetic condition is a methylmalonate and cobalamin (cbl; vitamin B12) metabolism genetically diverse condition. Isolated methylmalonic aciduria is observed in patients with partial, mut (-) or complete mut (0) mutations in the MUT gene producing enzyme deficiency. This form is just not B12 treatment responsive. A subset of patients with synthesis-default MUT coenzyme adenosylcobalamin (AdoCbl) are also affected by a number of forms of isolated methylmalonic aciduria and are classified in complementary groups: cblA (251100), caused by MMAA gene mutation (607481) at 4q31 chromosome, and cblB (251110), due to MMAB gene mutation (77568) at 12q24).[1-3]

CASE REPORT

The male newborn was born of a consanguineous marriage. His brain MRI brain showed high signal with

reduced diffusivity at the thalamocapsular region and hippocampi on both sides suggestive of post ictal edema or metabolic encephalopathy. He was suspected to be affected with inborn errors of metabolism in organic acidemia and has evaluated for pathogenic variations.

C3DC/C10 and C5DC/C3DC. Injection butyl ester analysis cannot distinguish between C3DC and C8OH by tandem mass spectrometry. Consequently, in some medium-chain metabolic diseases, the result of the C3DC

We collected samples for newborn screening from newborn babies in Tamil Nadu 48 hours following delivery. The AA/AC profile indicated malonyl carnitine elevation by tandem mass spectrometry as butyl esters (C3DC). Also anomalous were the associated ratios

might contain a contribution from C8OH. Further confirmatory testing for diagnosis was required. We conducted second-level DBS chromatography/tandem mass spectrometry, which was somewhat higher. Table 1 shows the results for this patient and the reference ranges.

Table 1: Results of the newborn screening using LC-MS/MS for amino acids and acylcarnitine profiling.

Analytes	Observed values (µmol/l)	Reference ranges (µmol/l)
Amino acids profiling		
Alanine	0.18	0.35-4.52
Arginine	6.09	0.37-33.10
Citrulline	0.92	0.40-8.45
Glutamate	0.93	0.12-3.45
Leucine/isoleucine/hydroxyproline	1.32	0.19-3.01
Methionine	0.71	0.30-3.14
Ornithine	0.5	0.30-3.89
Phenylalanine	0.99	0.28-3.15
Proline	0.82	0.26-3.00
Tyrosine	0.39	0.22-7.72
Valine	1.32	0.27-3.03
Acyl-carnitine profiling		
Free carnitine, C0	0.87	0.34-4.12
Acetyl carnitine, C2	0.19	0.17-4.27
Propionyl carnitine, C3	3.2	0.08-6.50
Propionyl carnitine/acetyl carnitine (C3/C2 ratio)	16.8	0.18-5.00
Malonyl carnitine, C3DC	8.72	0.00-4.55
Butyryl carnitine, C4	2.24	0.31-6.60
3-OH-isovalerylcarnitine, C5-OH	1.19	0.00-5.5
Tiglylcarnitine	1.43	0.00-12.86
Glutaryl carnitine, C5-DC	1.71	0.00-5.00
Hexanoyl carnitine, C6	2.75	0.00-6.00
Octanoyl carnitine, C8	2.13	0.33-8.22
Octenoylcarnitine, C8:1	1	0.00-14.38
Decanoylcarnitine, C10	1.25	0.31-5.54
C3DC/C10	6.9	<3.00
C5DC/C3DC	0.19	0.3-6.00
Dodecadienoylcarnitine, C10:2	0	0.00-8.75
Tetradecanoylcarnitine, C14	0.43	0.26-7.30
Hexadecanoylcarnitine, C16	0.25	0.00-2.72
3-OH-hexadecanoylcarnitine, C16-OH	0.63	0.02-5.63
Octadecanoylcarnitine	0.39	0.00-2.77
Octadecenoylcarnitine	0.23	0.00-2.77
Succinylacetone	0.97	0.46-2.87
Adenosine	0.8	0.00-6.7
2-deoxyadenosine	0.71	0.00-6.43
C26:0-lysophosphatidylcholine	0.78	0.29-2.69
Argininiosuccinic acid	1.65	0.00-9.01
Glutamic acid	0.38	0.26-3.80

At delivery, the newborn was asymptomatic and was released as normal vaginal delivery from the hospital. Based on the findings of NBS, the male newborn was recalled to hospital for evaluation and additional research on the basis of suspicion of an organic acid problem. Besides physiological jaundice, organic acidemia like hypoglycemia or metabolic acidosis did not have any clinical properties in the newborn. Blood results for glucose, renal function tests, electrolytes, liver function tests, lactate, ketone bodies and blood gases were not reduced to normal limits, save for modestly increased overall bilirubin.

Samples were collected for confirmatory testing and forwarded for processing to the Institute of Child Health and Hospital for Children, affiliated institute to Madras Medical College. The study of plasma acylcarnitine's verified the increase in malonyl carnitine. Organic urine acids revealed significantly higher malonic acid and 3-hydroxy-isobutyric aciduria with a modest rise in methylmalonic acid. Molecular testing of the MTO1 gene mutation c.1234A>G (p.Thr412Ala) by MedGenome-gene analysis was conducted.

The child's parents were married with consanguine. The newborn baby is now ten months old and regularly tracked at the metabolic clinic. It has typical milestones of growth and development. He receives 300 mg orally 3 times a day of L-carnitine additionally. The newborn is cared for by the dietician who started him three times daily on a formula with low long-chain triglyceride (LCT). Hypertrophic cardiomyopathy is monitored periodically by the cardiologist.

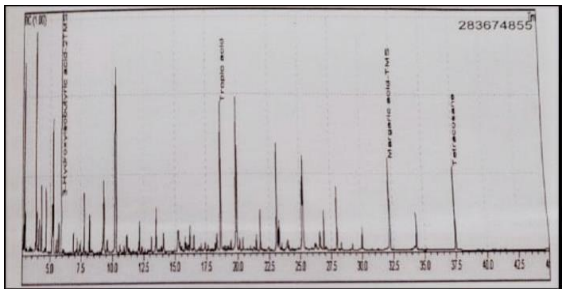

Figure 1: This GC-MS urinary organic profile shows the abnormal peak results in increased 3-hydroxy-isobutyric aciduria results in MMA patient.

Clinical exome sequencing results shown as variant 1: MTO+1 gene: a homozygous missense variation in exon 7 of the MTO gene (chr6:G.73480779A>G); Depth 108,that results in amino acid substitution of alanine for threonine at codon 412 (p.Thr412Ala; ENST00000415954.6). Missense variant in the nearby codon (c.1232C>T/p.Thr411ile, C.1402G>A.Ala428T). Variant 2: MMUT gene: a heterozygous missense variation in exon 7 in MMUT gene (chr6:g4944817T>C; depth 92×) that results in the aminoacid of glycine for glutamic acid at codon 448 (p.Glu448Gly) (Figure 1).

DISCUSSION

Methylmalonic acidemia (MMA) is a rare genetic disorder that affects the body's ability to break down certain proteins and fats. The condition is caused by mutations in genes that are involved in the metabolism of vitamin B12, which is essential for the proper functioning of the nervous system and the production of red blood cells. While there is no cure for MMA, treatment options such as dietary changes, vitamin B12 supplements, and medication can help manage symptoms and improve quality of life. Malonyl-CoA decarboxylase deficiency is a very rare condition.[4] By combining appropriate primary and secondary indicators, the AA/AC screen on DBS can detect rare inborn metabolic disorders. The AA/AC screen identifies patients with malonic aciduria who have abnormal levels of the related markers C3DC/C10 and C5DC/C3DC.[5] The AA/AC profile data may be uploaded and compared to normal populations and previously reported aberrant findings for certain metabolic disorders to confirm the suspicion of a specific metabolic problem.[6]

A subsequent DBS research also contributes to the validation of the first DBS findings. Confirmatory tests, such as plasma acylcarnitines, urine organic acids, and molecular testing, are necessary to establish the diagnosis and rule out the presence of other illnesses or interferences.[7] Moreover, the results of DBS testing can also inform treatment decisions and monitoring of disease progression. It is important for healthcare providers to consider all available diagnostic tools and collaborate with specialists to provide comprehensive care for patients with suspected metabolic disorders.[8]

Along with malonic acid, this condition results in a rise in methylmalonic acid. The amount of methylmalonic acid in urine is significantly more than the amount of malonic acid. Hypotonia, developmental delay, failure to thrive, hypoglycemia, and coma are all symptoms of this condition.[9] Certain individuals have microcephaly. MMA do not exhibit symptoms until they reach adulthood and suffer from neurological problems such as seizures, memory loss, cognitive decline, or psychiatric disorders. Malonyl-CoA decarboxylase catalyses the conversion of malonyl-CoA (a precursor to fatty acids and an oxidation inhibitor) to acetyl-CoA and CO2. Then shown that L-carnitine (100 mg/kg body weight) reduced malonic acid levels in urine in two case reports.[10]

We hypothesised that L-carnitine supplementation is critical in treating malonic aciduria patients because it increases beta-oxidation of fatty acids via CPT1, decreases malonyl-CoA levels, and avoids metabolic decompensation such as metabolic acidosis and hypoglycemia. Our newborn screening patient who was asymptomatic was taking L-carnitine supplements.[11] It was important to note that the dosage and duration of L-carnitine supplementation should be determined by a healthcare professional and regularly monitored to ensure its effectiveness. Additionally, further research was

needed to fully understand the long-term benefits and potential side effects of L-carnitine supplementation in malonic aciduria patients.[12]

His growth and developmental milestones had been fulfilled normally. They concluded that chronically increased malonic acid consumption may result in cardiomyopathy. Asymptomatic cardiomyopathy patients should be closely watched to ensure prompt diagnosis and therapy. At nine months, our patient's first echocardiogram indicated significant left ventricular dilatation. He was currently on an LCT-restricted diet. He may require an ACE inhibitor after a month of assessment. The patient's parents had been advised to monitor his diet and ensure that he receives adequate nutrition. Regular follow-up appointments with a paediatric cardiologist have also been scheduled to track his progress and adjust his treatment plan as needed. It is important to note that early detection and intervention can greatly improve outcomes for patients with cardiomyopathy. In addition, lifestyle modifications such as regular exercise and stress reduction techniques may also be recommended to support overall heart health.[13]

Combined methylmalonic acidemia and homocystinuria is a rare inherited disorder affecting intracellular cobalamin metabolism. It is crucial for high-risk couples to diagnose the disorder, as it can be life-threatening for offspring. Two infant deaths were likely due to cblC, and prenatal genetic testing was performed. Parental clinical exome sequencing revealed a heterozygous pathogenic variant in the mother's MMACHC gene and a nonsense variant in the father's MMACHC gene. The mother delivered a healthy baby, and the neonate did not show symptoms or signs of combined methylmalonic acidemia and homocystinuria after birth.[14]

In Taiwan, a nationwide newborn screening program for isolated methylmalonic acidemia (MMA)/propionic acidemia (PA) in neonatal has shown that patients with MMA mutase type and PA cases are at risk for death and neurodevelopmental disability. The study found that MMA patients have higher AST, ALT, and NH3 values, and lower pH values. Liver transplantation was successful in MMA/PA patients with LT, with reduced admission time, tube feeding ratio, and caregiver anxiety. The study suggests that MMA/PA patients with LT can survive and have improved DQ/IQ performance.[15]

NBS for propionic acidemia (PA) and isolated MMA in the Netherlands has limited evidence. A retrospective cohort study evaluated the clinical course of 76/83 patients diagnosed between 1979 and 2019. Five clinical outcome parameters were defined: adverse outcome of the first symptomatic phase, frequency of acute metabolic decompensations (AMD), cognitive function, mito-chondrial complications, and treatment-related complications. Results showed an adverse outcome in 46% of patients, similar in 5/9 sibling pairs and better in 4/9 pairs. The health gain of NBS for PA and MMA may be limited, with only a modest decrease in adverse outcomes due to the first symptomatic phase expected.[16]

Organic acidemias, such as propionic acidemia (PA) and MMA, can manifest in newborns, leading to metabolic acidosis, hyperlactatemia, and hyperammonemia. Early diagnosis and treatment have reduced neonatal mortality and improved survival. Liver transplantation (LT) has been proposed as a treatment modality to reduce metabolic decompensations. A retrospective chart review of nine individuals with PA or MMA who underwent LT and two with MMA who underwent LT and kidney transplantation showed a 100% survival rate at 1 and 5 years post-LT. Most subjects did not experience hyperammonemia or pancreatitis post-LT, and protein restriction may be indicated even after LT.[17]

Vitamin B12 metabolism alteration, either genetic or acquired, can lead to anemia, developmental regression, and neurologic damage. Early diagnosis and intervention are crucial. A study assessing the usefulness of the second-tier test, MMA, methylcitric acid (MCA), and homocysteine (Hcys), in newborn screening programs found that the percentage of cases with MMA above the cut-off levels was similar for both genetic and acquired conditions. However, the percentage of cases with increased levels of Hcys was higher in acquired conditions than in genetic disorders. MCA was high only in 5% of acquired conditions, while propionic acidemia was always high in all patients. The results support the inclusion of acquired vitamin B12 deficiency in newborn screening programs, as it is easily detectable and allows for corrective measures to avoid its consequences.[18]

CONCLUSION

This variant has been previously reported in ClinVar as a likely pathogenic mutation associated with Methylthioribose-1-phosphate dehydratase deficiency. This finding suggests that the patient may have this metabolic disorder and further confirmatory testing is recommended. Missense variant in the nearby codon (c.1232C>T/p.Thr411ile, C.1402G>A.Ala428T) were also reported in patients with the same disorder, supporting the pathogenicity of this variant. It is important to consider genetic counselling and early treatment for affected individuals to prevent potential complications. This variant has been reported in a patient with methylmalonic acidemia, which is consistent with the known association between MMUT gene mutations and this disorder. Genetic testing for family members of affected individuals may also be beneficial in identifying carriers and guiding reproductive decisions.

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