MAPLE SYRUP URINE DISEASE: AN UNCOMMON CAUSE FOR NEONATAL METABOLIC DISTRESS

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ABSTRACT

Maple Syrup Urine Disease is an autosomal recessive disorder caused by a deficiency in the activity of the branched-chain α -ketoacid dehydrogenase complex. This rare disorder represents one of the causes of acute neonatal illness which results in devastating disturbances of neurological development. On investigation of 1750 infants with neurological impairment for inborn errors of amino acid metabolism, 4 neonates with classical maple syrup urine disease were detected. These otherwise normal neonates presented in the first week after birth with seizures, lethargy and refusal of feeds, hypoglycemia and metabolic acidosis. The plasma and urine concentrations of the branched-chain amino acids were increased and there was ketoaciduria. Two of these neonates expired before specific treatment could be instituted. Routine biochemical screening of neonates with acute illness could unearth many cases of this rare inherited metabolic disease.

KEY WORDS: Leucine, isoleucine, valine, α -ketoacids, inherited metabolic disorder

INTRODUCTION

Maple syrup urine disease (MSUD) or branched-chain ketoaciduria is a heterogeneous, inherited disorder of the branched-chain amino acid metabolism caused by a deficiency in the activity of the branched-chain α -ketoacid dehydrogenase (BCKD) complex. The frequency of this panethnic disorder is 1 in 185,000 (1). In a screening of 98,256 newborns for aminoacidemias by thin-layer chromatography 11 cases of branched-chain aminoacidemias have been reported from South India (2). Although this disorder is rare it represents one of the causes of devastating disturbances of neurological development that is

potentially treatable. For the clinician the problem of recognition of this disease revolves round the paucity and non-specificity of the signs and symptoms and the non-availability of specialized laboratories which could give a confirmed diagnosis. If appropriate laboratory tests are not pursued, the diagnosis could be missed and this treatable disorder could go unrecognized. Further, failure to identify patients with this inborn error of metabolism obviates the possibilities of genetic counseling and prenatal diagnosis. Our aim was to detect and conclusively confirm the diagnosis of any inherited disorder of amino acid metabolism in newborns with seizures and other signs of acute neonatal illness and older children with

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neurological impairment for further appropriate management of these cases.

MATERIALS AND METHODS

1750 children who presented with seizures, lethargy, failure to thrive, recurrent vomiting, developmental delay or any other signs of neurological impairment, including 170 neonates with metabolic encephalopathy were investigated for amino acid disorders. A detailed medical history of each patient along with the relevant clinical findings were recorded. 3 ml of heparinised blood and 25 ml of fresh urine was collected. After routine qualitative screening tests for abnormal metabolites in the urine, the total amino acid concentration in the plasma and urine was estimated colorimetrically by the method of Goodwin using the reaction of 2,4-dinitrofluorobenzene with the α -amino groups in alkaline solution (3). The individual amino acids were separated by thin-layer chromatography on cellulose acetate plates using butanol-acetone-acetic acid and water as the solvent system in the ratio of 7:7:2:4. V/V The amino acids were visualized by heating the plate at 75° C for 3 min. after staining with ninhydrin. Whenever an abnormal amino acid profile was encountered, the concentrations of the individual amino acids were estimated by separation with a reverse-phase high-performance liquid chromatography (HPLC) using a C18 column after pre-column derivatization with o-phthalaldehyde (4).

RESULTS

Four cases of classical maple syrup urine disease were detected among the 1750 children investigated.

Case 1

A six-day old female child of second degree consanguineous parents presented with complaints of poor feeding, vomiting and lethargy. The baby had been delivered normally and was feeding well till she was five days old when she was noticed to gradually become inactive and refused to suck at the breast. On examination she was lethargic, hypertonic, rigid with severe opisthotonus. She was also noticed to have an abnormal odor. Routine biochemical investigation revealed low blood glucose levels of <30 mg/dl despite correction by IV fluids, and ammonia of 398 mg/dl (25-95 mg/dl). The patient also had metabolic acidosis with a bicarbonate level of 14 mEq/L. The sepsis work-up was negative and the cranial CT scan was normal. Urine screening for inherited metabolic disorders showed a strongly positive dinitrophenylhydrazine (DNPH) test for α -ketoacids and the presence of ketonuria was detected by strip test. A thin layer chromatogram of the plasma and urine amino acids showed increased leucine, isoleucine and valine (Fig 1). On quantitative estimation of the individual amino acids by HPLC, the concentration of leucine, isoleucine and valine were elevated (Table1). These findings along with ketoaciduria confirmed the presence of the classical form of maple syrup urine disease. The baby improved remarkably with peritoneal dialysis and was put on a low protein diet with thiamine supplementation. However, the infant was lost to follow-up.

Case 2

Another 9-day-old male neonate of second-degree consanguineous parents presented with lethargy, refusal of feeds and seizures.

Fig. 1. Thin-layer chromatogram of the plasma and urine amino acids in Maple syrup urine disease
Lane 1. Standards: G glycine, V valine, I isoleucine, Lane 2. Patient's urine, Lane 3. Control urine,
Lane 4. Patient's plasma and Lane 5. Control plasma

Physical examination revealed a flaccid child with a distinct odor. The deep tendon reflexes and Moro reflex were absent. There was no organomegaly. The routine blood chemistry showed the presence of hypoglycemia and metabolic acidosis. The DNPH test was found to be positive in the urine. Plasma and urine total amino acids were elevated and thin-layer chromatogram of the plasma and urine amino acids showed elevated levels of leucine, isoleucine and valine. Quantitative amino acid analysis done with a HPLC showed increased concentrations of leucine, isoleucine and valine in the blood confirming the diagnosis of MSUD. The baby expired within a day before specific treatment measures could be instituted.

Case 3

A 4-day-old male baby born to second degree consanguineous parents developed generalised tonic-clonic seizures lasting for 3-5 minutes and refusal of feeds. The baby was drowsy and an abnormal odor was noticed in the urine on examination. No other abnormality was noted. A repeated examination of the blood showed low glucose levels of 19mg/dl and 28mg/dl despite correction by intravenous fluids containing glucose. Mild metabolic acidosis was present. Since the sepsis work-up and cranial ultrasonogram was normal the child was investigated for a metabolic disorder. The DNPH

Table 1. Plasma and urine amino acid concentrations in the neonates with MSUD compared with normal neonates

Amino acid	Normal range	MSUD Case 1	MSUD Case 2	MSUD Case 3	MSUD Case 4
Plasma					
Total amino					
Acids (mmol/L)	2.5 - 3.7	12.1	14.0	7.1	15.6
Alanine (μmol/L)	131-710	71	87	53	42
Aspartate	20-129	7	4	8	4
Glutamine	376-709	143	40	240	101
Glycine	232-740	172	184	123	91
Histidine	10-138	11	10	15	10
Isoleucine	26-91	265	476	348	170
Leucine	48-160	417	462	452	391
Serine	99-395	81	107	68	34
Threonine	90-329	61	71	57	40
Valine	86-190	281	365	362	223
Urine					
Total amino acids					
(mmol/g creat)	upto 25	78.1	80.0	35.1	31.5
Alanine					
(μmol/g creat)	660-2160	174	323	1538	170
Glycine	2500-9700	1277	1711	657	2624
Histidine	700-2600	500	3130	280	350
Isoleucine	ND-53	148	1546	480	288
Leucine	26-220	1000	2480	1514	720\
Valine	26-230	741	1361	561	552

The other plasma and urine amino acids, arginine, glutamate, lysine, methionine, ornithine, phenylalanine, tyrosine and taurine were within normal limits in all four MSUD cases.

test was positive and the branched-chain aminoacids were elevated in blood and urine confirming the diagnosis of MSUD (Figs2&3). Although peritoneal dialysis was started immediately the baby expired.

Case 4

A 10-day-old female child of third degree consanguineous parents was admitted with complaints of decreased activity and refusal of

Fig. 2. HPLC pattern of the plasma amino acids in Maple syrup urine disease.

Fig. 3. HPLC pattern of the urine amino acids in Maple syrup urine disease.

feeds. A physical examination revealed a hypotonic drowsy neonate. Routine investigation did not reveal any abnormality except a mild metabolic acidosis. The sepsis screen was negative. However, urine showed the presence of ketoacids and elevated levels of leucine, isoleucine and valine was detected in the blood and urine on investigation for metabolic disorders confirming the diagnosis of MSUD. Appropriate treatment was instituted immediately. The baby improved remarkably and discharged after a month with an advise to restrict dietary intake of branched-chain amino acids by reducing protein intake to 1.5g/kg body weight/day, with a total calorie intake of 125C/kg body weight/day, and oral supplementation of thiamine at a dose of 10mg/day.

DISCUSSION

Maple Syrup Urine Disease (MSUD) was first described by Menkes in 1954 in four patients who died in the neonatal period and had odour strikingly reminiscent of maple syrup (5). In its classic form, MSUD is a fulminating neonatal neurological illness, characterised by anorexia and apathy by the end of the first week, soon followed by hypertonicity, opisthotonus and death if untreated (6). The abnormal odor of maple syrup is found in the urine, sweat and cerumen of most patients. All the four neonates with MSUD reported by us were otherwise healthy term infants but presented within a week with lethargy, vomiting, refusal of feeds and seizures. Many of these symptoms may be present in a child who suffers from an infection during the new born period. Hence, at the time an infectious etiology is being considered, it is equally important that metabolic causes be considered as well. Episodic variants

have been described with later onset and milder course, which have been referred to as 'intermittent branched-chain ketoaciduria'(7).

In untreated patients with classic MSUD, persistent and marked increases in the branched-chain amino acids, leucine, isoleucine and valine and their respective ketoacids, α -ketoisocaproate (KIC), α -keto- β -methylvalerate (KMV) and α -ketoisovalerate (KIV) are observed (2). The increases in the branched-chain amino acids in the plasma are frequently greater than 10-fold over normal. Leucine levels are usually higher than those of the other amino acids. The plasma concentrations of glucogenic amino acids have been reported to be markedly reduced. A low plasma alanine is consistently found during metabolic decompensation and is secondary to the consumption of alanine for reamination of increased branched-chain ketoacids (8). Other metabolites which accumulate, include L-alloisoleucine, which is produced from isoleucine by keto-enol tautomerization and transamination of α -keto- β -methylvaleric acid and α -hydroxyisovaleric acid, the hydroxy analogue of α -ketoisovaleric acid. N-acetylated BCAAs and N-lactyl BCAA have also been detected in the urine of MSUD subjects. Gas-chromatographic-mass spectroscopy of urine and plasma organic acids as their oxime-trimethylsilyl or quinoxalinol derivatives gives characteristic profiles (9).

Clinically significant fasting hypoglycemia has been observed in patients with classical MSUD (10). It was hypothesised that hypoglycemia was induced by elevated leucine which stimulated insulin (11). However, studies by Haymond et. al.(9) showed that the insulin levels were low and the concentrations of the glucogenic

amino acids like alanine were markedly reduced in the plasma of MSUD patients. Alanine concentrations increased rapidly as levels of BCAA decreased, following initiation of dietary therapy. Therefore, hypoglycemia appears to be due to reduced gluconeogenesis from amino acids. Hypoglycemia was present in three of the four cases detected by us. There was a marked reduction of alanine, and serine in the blood of all these patients confirming the hypothesis that hypoglycemia may be the result of a reduction of gluconeogenic amino acids. All other routine laboratory studies including the liver and renal function tests are all usually unremarkable except for the presence of metabolic acidosis. This finding was noted in all our cases also.

The biochemical effects associated with excessive quantities of branched-chain amino acids or ketoacids or both involve the metabolism of neurotransmitters and compounds important in energy metabolism, i.e., pyruvate and glucose, and the synthesis of proteins, myelin lipid and proteolipid proteins. The altered levels of neurotransmitters and the impaired energy metabolism may play an important role in many of the acute neurological deficits. Impairment of gamma-aminobutyric acid formation may occur because of impairment of glutamic acid decarboxylase, and perhaps because of diminished activity of the citric acid cycle and, therefore, α -glutarate formation (12). Serotonin may decrease primarily because of a disturbed uptake of 5-hydroxytryptophan, its immediate precursor (13). Energy metabolism may be altered in the presence of hypoglycemia and with decrease in the formation of acetyl Co A from pyruvate. Protein synthesis may be disturbed because of an altered transport of amino acids, as

well as a decrease in the aminoacyl-sRNA synthesis (14).

A simple biochemical screening test for the detection of MSUD is the dinitrophenylhydrazine (DNPH) test. The ketoacids present in the urine give a golden yellow precipitate with a few drops of the DNPH reagent (0.1% in 2 N HCl) due to phenylhydrazone formation (15). Another frequently used screening test is the Guthrie bacterial inhibition assay in which the growth of *Bacillus subtilis* 6051 by 4-azaleucine in a culture medium is reversed in the presence of increased leucine in the blood. Confirmation of the diagnosis is by quantitative analysis of the amino acid in the plasma and urine. The α -ketoacid derivatives of these aminoacids may be detected by organic acid analysis. The activity of the dehydrogenase enzyme is usually measured in lymphocytes, fibroblasts or lymphoblasts by measuring the conversion of ^{14}C -leucine to $^{14}\text{CO}_2$ (16).

The treatment of the acute state is aimed at quick removal of the branched-chain amino acids and their metabolites from the tissues and body fluids. Peritoneal dialysis is the most effective mode of therapy and should be instituted promptly. Attempts should be made to stop the patients

catabolic state by providing sufficient calories intravenously or orally. After recovery from acute state, treatment requires a low branched-chain amino acid diet. Synthetic formulas devoid of leucine, isoleucine and valine are commercially available. The patients must be carefully followed by monitoring plasma amino acid concentrations repeatedly. Some patients respond to treatment with large doses of thiamine. The mechanism of the response may involve decreased affinity of the mutant enzyme for thiamine pyrophosphate. Doses employed have ranged from 10-300 mg/day. All thiamine responsive patients have had residual branched-chain ketoacid dehydrogenase activity.

The outcome of optimal treatment before damaging ketoacidotic episodes can be normal brain development. However it has been observed that patients in whom treatment is initiated after 10 days of age rarely achieve normal intellect. Most cases of MSUD presenting in the neonatal period are lethal if specific therapy is not initiated immediately. Two of our four cases died even before treatment could be started. Specific diagnosis even in an infant whom death is inevitable is of great importance for genetic counseling of the family. Therefore every effort should be made to determine the diagnosis while the infant is alive.

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