

Etiology and outcome of inborn errors of metabolism

Shehla Choudhry,¹ Masood Khan,² Hani Akbar Rao,³ Anil Jalan,⁴ Ejaz Ahmed Khan⁵

Abstract

Objectives: To study the clinical presentation, diagnostic workup and outcome of children presenting with suspected inborn errors of metabolism.

Methods: The cross-sectional study was conducted at the Shifa International Hospital, Islamabad, and included all patients diagnosed with the condition between January 2006 and June 2011. Medical records of the patients were reviewed to collect the relevant data.

Results: A total of 10 patients underwent diagnostic work-up. Majority 7 (70%) were males and 6 (60%) presented in the neonatal age group. Seizures and coma were the commonest presentations (n=5; 50% each) followed by breathing difficulty (n=4; 40%) and vomiting (n=2; 20%). The commonest diagnoses were methyl malonic acidaemia (n=2; 20%), non-ketotic hyperglycinaemia (n=7; 10%), fructose 1,6 diphosphatase deficiency (n=1; 10%), and biotinidase deficiency (n=1; 10%). Mortality was high (n=5; 50%) and half of the survivors had severe neurological impairment.

Conclusion: The diagnosis of inborn errors of metabolism requires a high index of suspicion. These disorders have a high mortality and risk of long-term neurological disability.

Keywords: Inborn errors of metabolism, Methylmalonic acidaemia, Mitochondriopathy, Non-ketotic hyperglycinaemia, Fructose 1, 6 diphosphatase deficiency. (JPMA 63: 1112; 2013)

Introduction

Inborn errors of metabolism (IEMs) are rare as individual diseases, but as a group remain an important entity that present in paediatric age group.^{1,2} The timely diagnosis and early initiation of specific therapy may be life-saving in these patients. Many developing countries have succeeded in improving neuro-developmental outcome and overall survival in children with IEM by implementing evidence-based guidelines for neonatal screening, early diagnosis and treatment. Unfortunately, Pakistan lags far behind. This study aims to highlight IEM as an important clinical problem in our set-up, responsible for a number of neonatal and infant deaths which remain undiagnosed and unreported. We report our experience over the 5 years to highlight some of the problems of IEM in our setup.

Patients and Methods

The cross-sectional study was conducted at the Shifa International Hospital after approval from institutional ethics committee, and comprised all children diagnosed with IEM between January 2006 and June 2011 at the Paediatrics Department of the hospital.

.....
^{1,2,5}Department of Paediatrics, Shifa International Hospital, Islamabad, Pakistan, ³MEDICS Islamabad Hospital, Islamabad, Pakistan, ⁴NIRMAN, Mumbai, India.

Correspondence: Shehla Choudhry. Email: shehla126@yahoo.com

The children suspected of having IEM, who did not undergo detailed diagnostic workup were excluded from the study.

The enrolled patients initially underwent routine laboratory investigations, including cultures of blood, cerebrospinal fluid (CSF) and urine, electrolytes, renal and liver function tests, arterial blood gas analysis, serum ammonia, lactate, urine ketones and reducing sugar analysis. Neuroimaging was done as indicated in patients with seizures and coma to help in the diagnosis. Before starting the empirical therapy 4ml of blood (2ml in ethylene diaminetetraacetic acid [EDTA] and 2ml in sodium heparin), 2ml serum and 20ml urine were saved and refrigerated. As diagnostic facilities for IEM were not available at our setup, metabolic workup from abroad was offered to the parents and, if consented, samples were sent to a private Metabolic laboratory, Navi Mumbai Institute of Research In mental And Neurological Handicap (N.I.R.M.A.N), Mumbai, India. Thin layer chromatography (TLC) for amino acids (done in urine and plasma simultaneously to pick up any abnormality), sugars and oligosaccharides was performed. A combination of 20-25 tests, collectively referred to as Urine metabolic screening tests (MRST) was performed to screen for various metabolites in urine e.g ketones, glucose, ketoacids, ferric chloride, sulfites and nitrites. Usually galactose 1 phosphate uridylyl transferase (GALT)

enzyme, galactose and biotinidase enzyme were analysed using spectrophotometry or enzyme-linked immunosorbent assay (ELISA), while 17 hydroxyprogesterone was also performed by ELISA. High Pressure Liquid Chromatography (HPLC) for orotic acid, HPLC for purine and pyrimidines, tandem mass spectrometry for carnitine — acyl carnitine and amino acids, gas chromatography-mass spectrometry (GC-MS) of urine for organic acids and liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS) for amino acids were also performed to identify metabolic disorders, including those in carbohydrate, amino acids, fatty acid metabolism, urea cycle and organic acidemias. All the relevant data, including demographics, clinical presentation, laboratory investigations, specific metabolic workup done, treatment and outcome was recorded on a predesigned proforma. Data analysis was done using SPSS version 16.0. Frequency and percentage were

calculated for all the ordinal data.

Results

Ten patients underwent complete diagnostic workup of IEM. Majority (n=7; 70%) were males. Six (60%) presented in the neonatal age group (< 28 days) and all were born of consanguineous marriage. In 4(40%) patients, there was history of early neonatal deaths in previous siblings.

Seizures and coma were the commonest presentations (n=5; 50%) followed by breathing difficulty and acidotic breathing in 4 (40%), vomiting 3 (30%), reluctance to feed 3 (30%), and 2 (20%) patients had hepatomegaly (Tables-1a and 2a). The commonest laboratory findings included metabolic acidosis with elevated anion gap in 4 (40%), hyperammonaemia 4 (40%) and hypoglycaemia 3 (30%) (Tables-1b and 2b). The cerebrospinal fluid examination was normal and brain imaging (CT/MRI) revealed cerebral

Table-1a: Demographics, Clinical Features and Outcome in children with confirmed IEM.

Pt ID	Age/ Sex	Clinical Features	Duration of symptoms	Outcome
SIH-2	36 m/M	Fever, vomiting, acidotic breathing, hepatomegaly	2 days	At 4 years developmentally normal ¹ but episodic decompensation during intercurrent infections
SIH-3	4 d/ F	Lethargy, coma	3 days	Expired within 3 days of hospitalisation
SIH-7	11 d/M	Seizures	10 days	At 1 ½ years, healthy, developmentally normal ¹
SIH-8	3 d/M	Reluctance to feed, lethargy, acidosis	2 days	Expired within 4 days of hospitalisation
SIH-10	17 m/F	Vomiting, lethargy, coma	7 days	Expired within 5 days of hospitalisation

Pt (Patient), ID (Identification Number), SIH (Shifa International Hospital), m (Age in months), d (Age in days), F (Female), M (Male).

¹Developmental assessment was done according to Denver developmental screening tool. IEM: Inborn errors of metabolism.

Table-1b: Diagnostic work-up in children with confirmed IEM.

Pt ID	Initial Laboratory Workup ¹	Diagnostic Tests	Results	Final Diagnosis
SIH-2	Hypoglycaemia (20mg/dl) High Anion Gap (38)	GC-MS ² Urine reducing sugars	Glycerol excretion in urine Fructosuria	Fructose 1,6 diphosphatase deficiency ³
SIH-3	Normal	TLC-Amino acids (CSF, urine & plasma)	Raised Glycine in CSF (726µmol/L) and plasma (3112µmol/L) CSF/Plasma Glycine: 0.23 (Normal: 0.02-0.08)	NKHG ⁴
SIH-7	Normal	Biotinidase enzyme Carnitine/ acyl carnitine profile	3.13 nmol/ml/min (N 4.5-7.5) Low level of free (11.5 µmol/l), total (17µmol/l) & acyl carnitine (5.5µmol/l)	Partial Biotinidase deficiency
SIH-8	Hyperammonemia (800µg/dl) High Anion Gap (37)	Urine MMA C3 Acyl Carnitine C3/C16 ratio	11000 µmol/mol Cr (N < 15) 50µmol/L (N < 5) 27.3 (N < 3.5)	Methylmalonic acidemia
SIH-10	High Anion Gap (37)	Urine MMA C3 Acyl Carnitine C3/C16 ratio	4600µmol/mol Cr (N < 15) 27µmol/L (N < 5) 42 (N < 3.5)	Methylmalonic acidemia

IEM: Inborn errors of metabolism. MMA: Methyl Malonic acidemia. TLC: Thin layer chromatography. NKHG: Non-Ketotic hyperglycinaemia. CSF: Cerebrospinal fluid.

¹Initial Laboratory work up included Random Blood Sugar (N = 50-150 mg/dl), Serum Ammonia (N = 31-123 µg/dl), Plasma Lactic Acid (N = 4.5-19.8 mg/dl), Anion Gap (N = 12).

²GC-MS: Gas Chromatography Mass Spectrometry.

³Fructose 1,6 diphosphatase deficiency was also suspected on biochemical results. The enzyme analysis was not performed.

⁴Non Ketotic Hyperglycinaemia.

Table-2a: Demographics, Clinical Features and Outcome in children with suspected IEM.

Pt ID	Age/ Sex	Clinical Features	Duration of symptoms	Outcome
SIH-1	11 m/ F	Fever, vomiting, coma, seizures, hepatomegaly	6 days	Expired within 7 days of hospitalisation
SIH-4	17 d/ M	Seizures, coma	12 days	Expired within 5 days of hospitalisation
SIH-5	6 d/ M	Seizures, hypoglycaemia	4 days	At 2 years, microcephalic with global developmental delay ¹ and seizures
SIH-9	24 m/ M	Lethargy, reluctance to feed, coma	10 days	Developmental regression ¹ , in vegetative state now

Pt (Patient), ID (Identification Number), SIH (Shifa International Hospital), m (Age in months), d (Age in days), F (Female), M (Male).

¹Developmental assessment was done according to Denver developmental screening tool.

IEM: Inborn errors of metabolism.

Table-2b: Diagnostic work up in children with suspected IEM.

Pt ID	Initial Diagnostic Workup	Diagnostic Tests	Results	Final Diagnosis
SIH-1 ⁵	Hyperglycaemia (480mg/dl)	Quantitative amino acids (LC/MS/MS)	Alanine 1400 (120-600)	Mitochondriopathy ⁴
	Hyperammonaemia (1038µg/dl)	Serum Ketone	Positive	
	Raised Plasma Lactic Acid (48mg/dl)	GC-MS ¹ of urine	Elevated Lactate, Pyruvate, 3-hydroxybutyrate, Acetoacetate	
	High Anion Gap (34)	TMS ²	Low free carnitine 8.3 µmol/L (24.7-64.6) Normal Acyl carnitine profile	
SIH-4 ⁶	Hyperammonaemia (597µg/dl)	Urine reducing sugars	Present	Pyridoxine dependency/ NKHG ³ ?
		Biotinidase	Partial low (3.37 nmol/ml/min)	
		Organic acids-GC/MS	Elevated lactate	
SIH-5	Hypoglycaemia (22mg/dl)	Quantitative amino acid	Raised alanine & low arginine	Mitochondriopathy ⁴
	Hyperammonaemia (687µg/dl)	Carnitine/ Acyl Carnitine profile	Low level of free, total & acyl carnitine	
	High Anion Gap (33)	Organic acid GC/MS	Elevated lactate, Succinate, 2-hydroxyglutarate	
SIH-9	High Anion Gap (30)	Quantitative amino acids	Raised alanine & low arginine	Mitochondriopathy ⁴
		Carnitine/ Acyl Carnitine profile	Low level of free, total & acyl carnitine	
		Organic acid GC/MS	Elevated lactate, Succinate, 2-hydroxyglutarate	

¹GC-MS (Gas Chromatography Mass Spectrometry). ²TMS (Tandem Mass Spectrometry). ³NKHG (Non Ketotic Hyperglycinemia). ⁴Mitochondriopathies were suspected based on biochemical results. Further diagnostic work up for mitochondriopathies (muscle/ liver/ skin biopsy, enzyme analysis and DNA studies) could not be done. ⁵In patient SIH-1, elevated Lactate, Pyruvate, Acetoacetate, 3-OH Butyrate in GC-MS of urine as well as elevated Alanine in blood are strongly suggestive of mitochondriopathy. ⁶Pyridoxine dependency was suspected clinically as the patient had intractable seizures but patient did not survive long enough to undergo further work up including CSF Pilocolic acid.

LC/MS/MS: Liquid chromatography-Mass Spectrometry-Mass spectrometry.

oedema in 6 (60%) cases.

The confirmed diagnoses were methyl malonic acidaemia (MMA) in 2 (20%), non-ketotic hyperglycinaemia (NKHG) in 1 (10%), fructose 1, 6 diphosphatase deficiency in 1 (10%), and biotinidase deficiency in 1 (10%) (Table-1b). Three (30%) patients were suspected to have mitochondriopathy based on biochemical parameters which included elevated lactate in urine or plasma, elevated alanine in plasma amino acids, urine GC-MS showing elevation of one or more of the following compounds; Pyruvate, Succinate, Fumarate, Citrate and Aconitate (a metabolite in Krebs' cycle). Further specific typing of mitochondriopathy was not possible due to lack of appropriate facilities. No clue to diagnosis could be reached in a baby who presented with intractable seizures. He was worked up for NKHG/pyridoxine dependency and needed CSF glycine levels for confirmation but unfortunately died and lumbar

puncture could not be done. Another baby who presented with neonatal seizures, underwent full diagnostic work-up, but was ultimately diagnosed as West Syndrome. After saving the blood and urine samples for diagnostic evaluation, all the patients received empirical therapy as follows;

Oral Carnitine: 50-100 mg/kg/day divided 8 hourly; Inj. Vitamin B12 500µg intramuscularly once daily; Tab/Syp. Biotin 5mg 4 times daily; Vit B1 (Thiamine) 50mg (oral or intravenous); Vit E 15 mg/day.

The babies were also provided supportive care, including nil by mouth till stabilisation of vital signs, maintenance of intravenous fluids (comprising 10% dextrose and electrolytes), broad-spectrum antibiotics, blood product transfusions along with correction of dehydration, acidosis, hypo/hyperglycaemia and electrolyte imbalance as indicated. Five (50%) patients required mechanical

ventilation. Body fluid cultures were negative. Mortality was high (n=5; 50%) and half of the survivors had severe neurological impairment.

Discussion

Inborn errors of metabolism (IEMs), a group of genetic disorders, affect various biochemical pathways in the body. They usually present in the neonatal period or infancy, but can present in childhood or even later. Early diagnosis followed by initiation of disease-specific management may help improve survival and neurological outcome in these patients.

Studies worldwide have reported varied incidence of IEMs. In British Columbia, IEMs have been reported in 40 cases/100,000 live births¹ and an even higher incidence of 150 cases/100,000 live births has been reported in Saudi population.² However, the data for Pakistani population is limited. At the National Institute of Child Health, Karachi, IEMs were confirmed in 16/ 62 (26%) cases and two-thirds of them had organic acidemias. Respiratory distress and developmental delay were the commonest presentations.³ A study screened 2,000 children for IEM by paper chromatography of urine and identified two sisters with alkaptonuria.⁴ At Mayo Hospital, Lahore, one case of alkaptonuria and three siblings with mental retardation and aminoaciduria were detected from among 2,000 children screened.⁵ These studies show that IEM, though undiagnosed, are not uncommon in our set-up. Among our patients, diagnosis was confirmed in 5 out of 10 who underwent diagnostic testing for IEM. Methylmalonic academia (MMA) was the commonest disorder identified.

Majority of the IEMs have an autosomal recessive pattern of inheritance. In our population, with about 50% of marriages taking place among first cousins, a high incidence of metabolic/genetic disorders has been reported.⁶ In our study, all the babies were born of consanguineous marriages. But it is important to highlight that IEMs should be considered in families without history of consanguinity, if clinical presentation is strongly suggestive.

Patients with IEM usually present with non-specific symptoms and signs including breathing difficulty, fever, vomiting, lethargy, seizures and coma. IEMs are also a common cause of developmental delay and mental retardation. A study on 285 Chinese children with neurodevelopmental disabilities showed that IEM constitute 36% of all the cases.⁷ In our subjects, coma and seizures were the commonest presentations followed by vomiting and breathing difficulty resulting from metabolic acidosis.

The introduction of GC-MS, and tandem mass spectrometry (MS-MS) substantially helped in determining the etiology of neurodevelopmental disabilities.⁷ The same techniques were employed to diagnose our patients.

Asymptomatic neonates with positive newborn screening results require further evaluation and, if needed, initiation of disease-specific management. In Austria, eight years' experience with MS/MS for IEM revealed an overall prevalence of 1:2,855 with amino acidemias being the commonest (1:4,980).⁸ Effective implementation of newborn screening programme in some centres in India has resulted in early interventions in patients with IEM, thus preventing death and disability and has also helped in prenatal diagnosis in the next pregnancies.⁹ In Pakistan, there is no nationwide neonatal screening programme for IEM to date and the diagnostic facilities are quite limited. Clues to probable diagnosis can be gauged by initial screening tests but no diagnostic tests are available. Over the last few years, we have established liaison with N.I.R.M.A.N, a metabolic centre in Mumbai, India, to facilitate the process. Families with affected babies are counselled to send the samples abroad for workup. However, high cost and poor ultimate outcome discourage most of them.

For patients with suspected or known IEMs, successful emergency treatment depends on prompt institution of therapy aimed at metabolic stabilisation.

Recent studies show that early treatment results in significant reduction in oxidative damage in patients with organic acidemias and L-Carnitine supplementation may be helpful.⁸ Other treatment modalities include use of co-enzyme Q, pyridoxine, biotin, Vitamin E along with correction of acidosis with sodium bicarbonate supplementation and management of hyperammonaemia with sodium benzoate, sodium phenylacetate and arginine as indicated. Haemodialysis has also traditionally been used in the acute management of children with IEM. A study demonstrated that high-volume haemofiltration can offer an alternative way to effectively remove small molecules in affected patients.¹⁰ In our hospital, we have been managing IEM with full supportive care comprising intravenous fluids, correction of blood glucose levels and electrolyte abnormalities, correction of metabolic acidosis and hyperammonemia. The empirical therapy that we administer includes a cocktail of carnitine, biotin, vitamin B12 and vitamin E and Co enzyme Q. There was 50% survival but half of the survivors were neurologically impaired.

It should be noted that early diagnosis and prompt initiation of supportive and specific therapy is life-saving and most important determinant of outcome in these children.

Conclusion

There should be a high index of suspicion for IEM in infants and children with seizures, coma, acidotic breathing, positive family history of earlier deaths in siblings, acute or recurrent acidosis and hyperammonaemia. MMA seems to be a common IEM in our environment. Mortality despite supportive therapy remains high.

References

1. Applegarth DA, Toone JR, Lowry RB. Incidence of inborn errors of metabolism in British Columbia, 1969-1996. *Pediatrics* 2000; 105: e10
2. Moammar H, Cheriyan G, Mathew R, Al-Sannaa N. Incidence and patterns of inborn errors of metabolism in the Eastern Province of Saudi Arabia, 1983-2008. *Ann Saudi Med* 2010; 30: 271-7.
3. Satwani H, Raza J, Hanai J, Nomachi S. Prevalence of selected disorders of inborn errors of metabolism in suspected cases at a tertiary care hospital in Karachi. *J Pak Med Assoc* 2009; 59: 815-9.
4. Nafees M, Muazzam M. Alkaptonuria: an inborn error of amino acid metabolism. *Ann King Edward Med Uni* 2009; 14 :68-71.
5. Nafees M, Muazzam M. Inborn errors of amino acid and carbohydrate metabolism. *Professional Med J* 2007; 14: 512-8.
6. Bittles AH, Black ML. Evolution in health and medicine Sackler colloquium: consanguinity, human evolution, and complex diseases. *Proc Natl Acad Sci U S A*. 2010; 107 (Suppl 1): 1779-86.
7. Guo L, Li BX, Deng M, Wen F, Jiang JH, Tan YQ, et al. Etiological analysis of neurodevelopmental disabilities: single-center eight-year clinical experience in South China. *J Biomed Biotechnol* 2011; 2011: Article ID 318616. doi:10.1155/2011/318616.
8. Kasper DC, Ratschmann R, Metz TF, Mechtler TP, Möslinger D, Konstantopoulou V, et al. The national Austrian newborn screening program - eight years experience with mass spectrometry. Past, present, and future goals. *Wien Klin Wochenschr* 2010; 122: 607-13.
9. Jalan AB. Neonatal screening for inborn errors of metabolism. In: Jalan AB. *Neonatal Metabolic and Genetic Disorders*. Vol 1. 2000: 1-9.
10. Lai YC, Huang HP, Tsai IJ, Tsau YK. High-volume continuous venovenous hemofiltration as an effective therapy for acute management of inborn errors of metabolism in young children. *Blood Purif* 2007; 25: 303-8.