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ORIGINAL ARTICLE

Selective Screening For Inborn Errors Of Metabolism In Children: Single Centre Experience From Karnataka

JAILKHANI R, PATIL VS., LAXMAN HB, SHIVASHANKARA AR, KULKARNI SP,RAVINDRA MS

ABSTRACT

A large number of inborn errors of metabolism (IEM) in children remain undetected in India due to lack of investigative facilities and economic restraints. We screened 50 children presenting with neurological and metabolic problems at a tertiary level teaching hospital in Karnataka for inborn errors of metabolism using a standard protocol. There was male preponderance of cases (75%). The commonest clinical presentation was convulsions (30%) followed by metabolic acidosis (15%). 15% of the cases showed history of sibling deaths. We have come across four interesting cases in the course of our study – Phenylketonuria, Methyl malonic aciduria, Mucopolysaccharidosis and Branched Chain aminoaciduria, which will be presented in detail in our paper.

Key words: Inborn errors of metabolism, Phenylketonuria, Urine screening tests for IEM, Mucopolysaccharidosis, Methylmalonic aciduria.

Corresponding Author:
Dr.Vidya.S.Patil
Email: vidyashankar02@yahoo.co.in

Introduction

Inborn errors of metabolism (IEM) are a heterogeneous group of disorders caused due to single gene defect which may manifest immediately after birth or within few days or weeks after birth[1]. Most of the IEM result from defects in any of the key enzymes of various metabolic pathways,leading to accumulation of compounds which follow an alternative pathway of metabolism, resulting in the production of toxic metabolites and deficiency of biologically important compounds[2].

Individually, IEM present variable incidences, but as a whole, they present a cumulative frequency of 1:500 newborns[3] Among children with mental retardation it was reported that 5.75% of them were due to inherited metabolic disorders[4], common disorders reported are mucopolysaccharidosis, Wilson's disease, glycogen storage disease and Galactosemia[5]. Verma (2000) reported that amongst 24 million annual births, 20,800 had metabolic disorders. Screening of newborns for amino acid disorders showed few disorders to be commonly occurring – Tyrosinemia, Alkaptonuria, Maple syrup urine disease[5].

Most IEM are inherited as autosomal recessive traits. Hence a history of parental consanguinity and/or sibling deaths should increase the suspicion of IEM. IEM causing clinical manifestations in the neonatal period are usually severe and often lethal. The newborn baby with IEM may present with neurologic or metabolic disturbance like – poor feeding, lethargy, failure to thrive, seizures, apnea, drowsiness, coma and later on there may be gross delay in the development of milestones. Sepsis often accompanies neonatal IEM and may confound further diagnosis[6].

Strong suspicion of IEM can be done if the child presents with any of the following features – parental consanguinity, sibling deaths, positive family history of similar illness/ deaths, convulsions, regression of milestones,mental retardation, ketosis, acidosis, persistent hypoglycemia, jaundice, organomegaly, dysmorphic features or coarse facial features, unusual odour to urine[6],[7].

Early diagnosis of the condition by various laboratory tests and initiation of prompt therapy is very much essential in order to prevent lethal complications which are irreversible. Various simple preliminary laboratory tests can aid in diagnosis of IEM which can be further confirmed by advanced diagnostic techniques. In our laboratory setup,we perform preliminary urine screening for IEM from the newborns presenting with either neurological or metabolic disturbances. Our hospital-based project aims to screen and diagnose IEM among these children.

Material and Methods

The present study was carried out at S.D.M College of Medical Sciences and Hospital, Dharwad, Karnataka, India. Neonates, infants and children presenting with either metabolic or neurological disturbances, convulsions, mental retardation, persistent vomiting, jaundice, organomegaly, dysmorphic skeletal features, regression of milestones, unusual odour of urine were included in the study. Detailed history regarding parental consanguinity and sibling deaths, birth history and postnatal development of the child was recorded.

Institutional ethical clearance was obtained for the study and informed consent was taken from the guardian of the child.

Samples were obtained from the Department of Pediatrics, S.D.M college of Medical Sciences and Hospital, Dharwad, and also from Bidari's Ashwini Institute of Child health and Research center, Bijapur, Karnataka, India and other local pediatricians. 50 children were included in the study among which 35 were males and 15 were females. 25 out of these were neonates, 11 were infants and 14 were more than one year of age.

25 ml of random sample of urine was collected from each patient in a sterile plastic container containing 0.5 ml of 6N HCl as preservative. Sample was centrifuged at 5000rpm for 15 minutes and supernatant was analysed for all physical and chemical parameters. Urine sample was screened for any physical variations in colour, odour, appearance, pH, specific gravity. Urine dipstick analysis was performed to detect the presence of Glucose, Proteins, Blood, Urobilinogen, Bilirubin, Ketone bodies.

Further, specific chemical tests were performed to screen for the presence of specific metabolites in urine in few commonly occurring IEM [Table/Fig 1][8].

(Table/fig 1) –Screening Tests For The Presence Of Specific Substances/ Metabolites In Urine

Test Name	Substances Showing Positive Tests
Benedicts test	Reducing substances particularly sugars like galactose, fructose, glucose
Sulphosalicylic acid test	Positive for Proteins
Rotheras test	Positive for Ketone bodies
Ferric chloride test	Positive in Phenylketonuria, Tyrosinemia, MSUD, Histidinemia, Alkaptonuria
Dinitriphenylhydrazine (DNPH) test	Organic acids (α keto acids)
Nitrosonaphthol test	Positive in Tyrosinuria
Cyanide nitroprusside test	Positive in Cystinuria and Homocystinuria
Ammoniacal silver nitrate test	Positive in Homocystinuria
P- Nitroaniline test	Positive in Methylmalonic aciduria
Toluidine blue spot test and Cetyl pyridinium chloride (CPC) citrate turbidity test	Positive in Mucopolysaccharidosis
Test for porphobilinogen	Presence of porphobilinogen

Confirmatory tests like Paper chromatography[9] and Thin layer chromatography[10] were done to detect the presence of aminoacidurias.

Additional laboratory investigations done which aided in the diagnosis of IEM are - Arterial blood gas analysis, Blood lactate, Pyruvate and Ammonia, Liver function tests, Serum electrolytes, Blood urea and serum creatinine, Urine protein electrophoresis and Biopsy and histopathology of tissue samples.

After performing all these preliminary laboratory investigations, any case of IEM diagnosed can be confirmed further in the referral centers by Tandem Mass spectrometry, a sophisticated instrument which can provide specific diagnosis by giving the exact amount of the abnormal metabolite seen in specific disorders.

Results and Discussion

Urine samples from 50 selected cases were screened for IEM during the study period of 20 months. Among the cases screened,

males were predominant (75%) and 50% of them were in the neonatal age group.

Analysis of the data revealed that the commonest clinical presentations among the study subjects were convulsions (30%), delayed milestones, metabolic acidosis and hypoglycemia (15%) followed by jaundice and organomegaly (10%) and least common findings were coarse facial features and skeletal deformities (5%). Family history showed sibling deaths in 15% of the cases [Table/Fig 2]

(Table/Fig 2) Data showing the commonest clinical presentations among the subjects screened for IEM

Clinical presentation	Number of cases
Total number of cases	50
Males	35
Females	15
History of Convulsions	15
Delayed milestones	8
Metabolic acidosis	8
Persistent hypoglycemia	8
Jaundice and organomegaly	5
Coarse facial features	3
Skeletal deformities	3
History of sibling deaths	7

During the course of our study, we have come across four interesting cases having IEM based on preliminary laboratory tests, clinical presentations and family history. Few of the cases showed non specific positive laboratory findings which did not correlate with the clinical scenario of the patient, like reducing sugars, proteins, blood, bilirubin, tyrosine which were later considered to be false positive due to interactions with drugs or other interfering substances. Here we are presenting four cases which were diagnosed to have IEM by preliminary screening tests.

Many of the Inborn Errors of Metabolism, including urea cycle defects, organic acidemias, and certain disorders of amino acid metabolism, present in young infant with symptoms of an acute or chronic metabolic encephalopathy[11]. In neonates, urea cycle defects and organic acidemias are the primary cause of hyperammonemia, where as in older infant, fatty acid oxidation defects may be considered. Hypoglycemia may be a predominant finding in glycogen storage disorders, defects in gluconeogenesis and fatty acid oxidation defects[12]. Jaundice or other evidence of hepatic dysfunction is the mode of presentation in galactosemia, hereditary tyrosinemia, neonatal hemochromatosis and a number of other conditions. Therefore, appropriate laboratory evaluation for metabolic disorders is to be done in those infants exhibiting these findings[11].

Phenylketonuria (PKU)

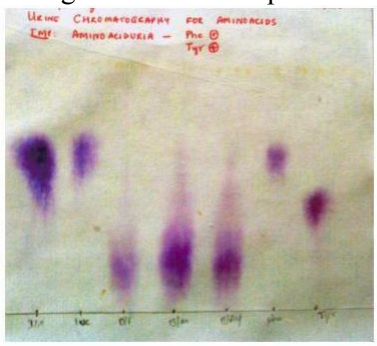
PKU is an autosomal recessive disorder most commonly caused by mutation in the gene coding for phenylalanine hydroxylase, an enzyme responsible for conversion of phenylalanine to tyrosine. In the absence of this main reaction, alternate pathways will be predominant and by products like phenylpyruvate and phenyllactate are produced in abundance. High plasma and urinary levels of phenylalanine, and its bye products, is seen. The incidence of this disorder is 1 in 10,000 live births[13].

One year old male child of a non consanguineous couple presented with history of delayed milestones and hyperirritability since birth. The child did not cry immediately after birth. The child was treated for Hypoxic Ischemic Encephalopathy stage – 1 with subtle seizures. Later the baby became lethargic with decreased activity and no demand for feeds. The child was noted to have delayed milestones like - no head control, no rolling, no grasping reflex, no babbling or cooing. There was no history of convulsions or vomiting. Mother noticed change in the colour of hairs (Golden blonde hairs) since last 2 months. No history of any peculiar odour of urine. There is a family history of mental retardation, but no history of either convulsions or albinism.

Examination revealed that the child was not interested in surroundings, had frequent myoclonic jerks with drooling of saliva. There was gross microcephaly (Head circumference – 37cm) with partial

head control, blonde hairs and puffy cheek. Overall motor, language and social developmental delay was noted. There was spasticity of lower limbs, brisk reflexes, extensor plantar reflex and visual inattention. With all these findings the child was clinically diagnosed to have Cerebral palsy with diplegia with delayed milestones (mental retardation) and myoclonic jerks**.**

Urine sample of the child was screened for IEM to rule out PKU in view of blonde hairs and mental retardation. Urine chromatography for aminoacidurias revealed increased excretion of Phenylalanine and Tyrosine which was suggestive of Phenylketonuria **[Table/Fig 3]**. Further confirmation of the case should be done by measuring blood phenylalanine levels using Tandem Mass spectrometry.



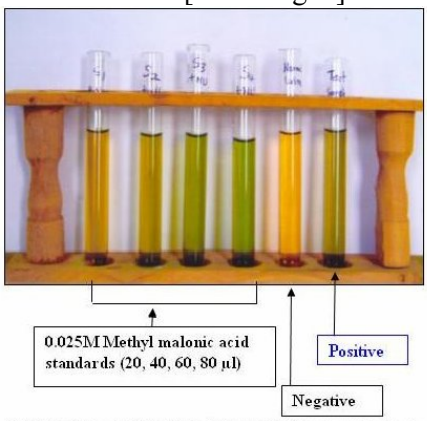
(Table/Fig 3) Urine chromatogram showing excretion of phenylalanine

Methyl Malonic Aciduria

Methylmalonicaciduria results from inherited autosomal recessive deficiency of methyl malonyl CoA mutase. Isolated deficiency of this enzyme results from mutations at the apomutase locis, and at two loci coding for deoxyadenosyl cobalamin (adocbl). Adocbl is the coenzyme for the reaction catalysed by methyl malonyl CoA mutase which converts methyl malonyl CoA to succinyl coA. Combined deficiency of methyl malonyl CoA mutase, methionine synthase (reaction in which homocysteine is converted to methionine with methyl cobalamin as coenzyme), and intracellular pathways for synthesis of coenzyme forms of cobalamin, also occur[14].

A four days old male infant was born to a second degree consanguineous couple. The antenatal period and the delivery of the baby were uncomplicated. The child cried immediately after birth and apparently looked healthy. The child was brought to the pediatrics referral centre after 16-18 hours of birth, when the baby stopped feeding at breast, was breathing hurriedly and then became drowsy. There was hypoglycemia on admission with blood sugar level of 47mg/dl. Blood sample was sent for Arterial Blood Gas (ABG) analysis and the baby was immediately taken to NICU. Laboratory investigations revealed wide anion gap metabolic acidosis. ABG analysis showed pH – 7.28, HCO3 – 6.5 mmol/L, Anion gap – 25 mmol/L, O2 saturation – 75%, pO2 - 65 mmHg, pCO2 – 13.6 mmHg, total CO2 content – 7 mmol/L. The child was immediately put on intravenous fluids (10% dextrose and sodium bicarbonate) and mechanical ventilation because of the impending respiratory failure. A sepsis workup was done and antibiotics were given.

History regarding the siblings revealed that there was death of 2 siblings after 4 days of birth. With the suspicion of inborn error especially organic acidemias, urine sample was sent to our laboratory for screening for IEM. Preliminary urine screening tests for IEM revealed a strong positive test for the presence of Methyl malonic aciduria [Table/Fig 4].



(Table /Fig 4) Test showing positive for Methylmalonic aciduria

There was no aminoaciduria on performing urine chromatography. The plasma ammonia levels were high. All these clinical and preliminary screening findings strongly favour the diagnosis of Methyl Malonic Aciduria. Further

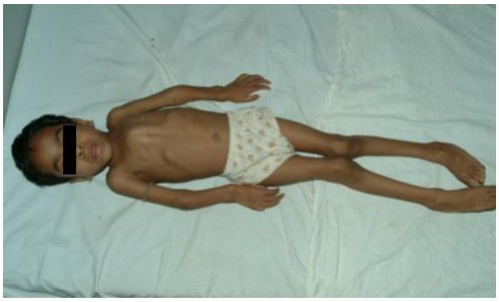
confirmation of the condition is to be done by quantitative estimation of methylmalonic acid in blood along with all other metabolites such as propionyl glycine, hydroxypropionate, propionylcarnitine etc, by Tandem MS or Gas chromatography – mass spectrometric analysis.

On day 3 , the child showed some improvement with RBS – 73 mg/dl, pH – 7.35, HCO3 – 8.3 mmol/L, anion gap – 20 mmol/L, O2 saturation- 97%, pO2 - 85 mmHg, pCO2 – 14.8 mmHg, Total CO2 content – 9 mmol/L. But the condition of the baby worsened on day 4 and it collapsed on day 5 due to metabolic acidosis and respiratory failure.

Mucopolysaccharidosis

MPS are autosomal recessive deficiencies of enzymes that degrade glycosaminoglycans. MPS uncommonly exhibit any clinical abnormalities in the first few months of infancy. Newborns with these syndromes present with coarse facial features, skeletal abnormalities and hepatosplenomegaly[15].

A 7 year old female child presented to the pediatric out patient department with the complaints of inability to walk since last 2 months. She had weakness in all the limbs and inability to move. She had bony abnormalities since birth. She had normal bladder and bowel control. She was short statured weighing 6 Kg and 87 cm tall with head circumference of 47 cm. General examination revealed mild pallor. No icterus/ edema/ lymphadenopathy was noted. Vital signs were normal. There was enlargement of the wrist joint and chest deformity (pectus carinatum) was present. CNS examination showed normal higher mental functions, low but normal speech. There was generalised hypotonia. Plantar reflex was normal. Lordosis of the lumbar spine was observed [Table/Fig 5].

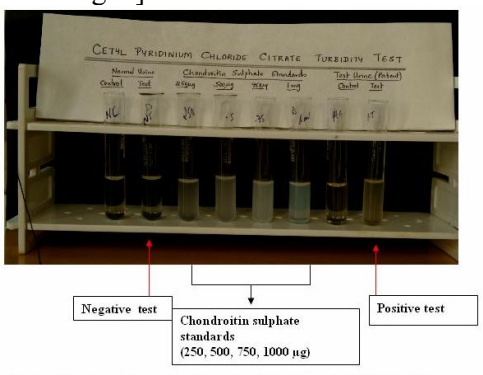


(Table/ Fig 5) Child with MPS showing Pectus carinatum, enlarged wrist and short stature

Ophthalmic examination showed cloudy cornea with pupils, lens and fundus being normal.

X-ray chest showed horizontally placed ribs which were spatula shaped. Skull bones were thick. Short bullet shaped phalanges were noted. X-ray of the hip (Iliac bone) showed shallow acetabulum and long bones showed osteoporotic changes. The child was given the provisional clinical diagnosis of mucoploysaccharidosis and urine sample was sent for screening for IEM.

Screening of the urine sample for IEM showed strong positive test for Mucopolysaccharidosis. Toluidine blue spot test and Cetyl pyridinium chloride citrate turbidity test showed positive results suggesting the presence of Mucopolysaccharidosis (MPS) [Table/Fig 6].



(Table/Fig 6) Cetyl pyridinium chloride citrate turbidity test for mucopolysaccharides showing turbidity in patient's urine sample

Urine chromatography for aminoacids revealed no aminoaciduria. All other tests for abnormal metabolites were negative. Correlation with the clinical picture gave the possibility of MPS Type IV disease. Further confirmation of the condition needs to be done by quantitative analysis of the enzyme deficient in this condition. Since false positive mucopolysaccharide

test results are commonly observed in neonates, definitive diagnosis should be made by appropriate biochemical studies on leucocytes or cultured skin fibroblasts[12].

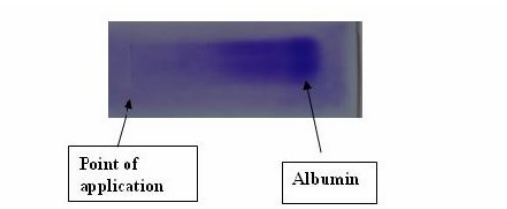
Branched Chain Aminoaciduria (Maple syrup urine disease)

MSUD is caused by a deficiency in the activity of branched chain alpha-keto acid dehydrogenase complex. This metabolic block results in the accumulation of branched chain amino acids- leucine, isoleucine and valine , and their keto acids. Classic MSUD has a neonatal onset with encephalopathy, and is the most severe and common form of MSUD. Its incidence world-wide is 1: 185,000[16].

A 4 day old male child of a consanguineous couple had uneventful antenatal period and birth history. The child was normal immediately after birth. After 3 days the child developed vomiting and difficulty in feeding. The child was breathing irregularly and became lethargic later on with failure to thrive. Arterial blood gas analysis showed metabolic acidosis. Ultrasonography of the abdomen showed bilateral renal grade III parenchymal changes. The child was taken to NICU where he was put on I.V fluids and mechanical ventilation. Any chance of neonatal sepsis was ruled out.

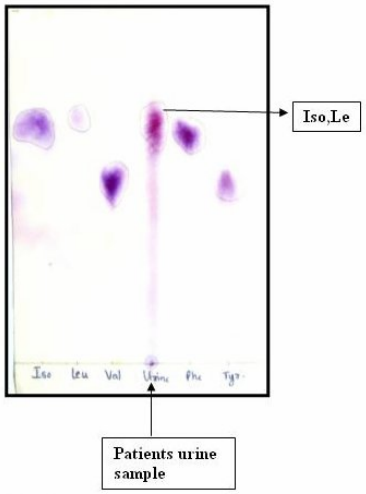
Family history revealed death of 2 siblings of the child after 1 to 2 weeks of birth, the cause of which was not known. Hence with the strong suspicion of inborn metabolic disorder, urine sample was sent for screening of IEM.

Urine sample was turbid, yellow and foul smelling. Test for proteins showed significant proteinuria, which was confirmed by performing urine electrophoresis for proteins. It showed a thick band of albumin and also quantitative estimation showed the presence of 3.3 g/dl of proteins to be present in urine [Table/Fig 7].



(Table/Fig 7) Urine Electrophoresis showing Thick Albumin band

Further DNPH test for ketoacids in urine was strongly positive. Urine paper chromatography for amino acids showed Branched Chain Aminoaciduria (Isoleucine, Leucine) which was confirmed by Thin layer chromatography [Table/Fig 8]. Further confirmation of branched chain aminoaciduria should be confirmed by quantitative analysis by Tandem MS.



(Table/Fig 8) Urine chromatogram showing branched chain aminoaciduria

Routine laboratory investigations, chemical tests for abnormal metabolites in urine, and paper and thin layer chromatography are sufficient for screening and provisional diagnosis of IEM. However, confirmation of diagnosis of IEM needs further procedures, like estimation of metabolites and enzymes in body fluids by Tandem mass spectrometry and High performance liquid chromatography.

Conclusion

There is a need to create awareness in the general population on the ill-effects of IEM and the need to prevent them. Screening programmes and prenatal diagnosis of IEM will go a long way in prevention of IEM, and in genetic

counseling. This would benefit the society as a whole in reducing and preventing psycho-social burden of the medical consequences due to IEM. The concept that "genetic disorders are very difficult to diagnose, and if diagnosed, it is impossible to treat" no more stands.

Abbreviations

IEM – Inborn errors of metabolism
PKU – Phenylketonuria
MSUD – Maple syrup urine disease
MPS - Mucopolysaccharidosis
TLC – Thin layer chromatography
Tandem MS – Tandem mass spectrometry

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