

Prevalence of selected disorders of inborn errors of metabolism in suspected cases at a Tertiary Care Hospital in Karachi

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Abstract

Objective: To study the prevalence of selected disorders of inborn errors of metabolism at a tertiary care hospital in Karachi by performing selective screening of high risk clinically suspected individuals.

Methods: Cross sectional comparative study, was done at the Paediatric Endocrine Unit 2 of National Institute of Child Health Karachi in collaboration with Sapporo City Institute of Public Health, Japan. Sixty-two children age < 1 month-10 years meeting the inclusion criteria (Undiagnosed family history of similar illness or deaths, history of recurrent episodes of severe or persistent vomiting for which no infection or surgical cause was found and history of undiagnosed neurological symptoms and developmental delay) were enrolled in the study. Routine workup of inborn errors of metabolism was done in each child and their dried blood samples (DBS) and dried urine samples (DUS) were send to IEM Selective Screening Unit Japan. SPSS version 10 was used to derive results and p-value of <0.05 was taken as statistically significant.

Results: Out of 62 children, sixteen children (9 boys and 7 girls) were positive for inborn errors of metabolism (IEM). Respiratory distress (p=0.042) and developmental delay (p=0.048) were found to be the most common clinical presentations in our children .Out of 16 children with positive results, 14 children had history of death of siblings with similar complaints (p=0.027). Consanguineous marriage was reported in 13 children. Among children with positive results 10(62.5%) had organic acidemias, 1 (6.2%) had Ornithine Transcarbamylase (OTC) deficiency (Urea cycle defect) and 5(31.2%) had congenital lactic acidemias.

Conclusion: Significant number of positive cases were seen in our series of patients, establishing the fact that IEM is prevalent in our population, though undiagnosed. Further such studies are needed on our side in future to determine incidence of metabolic disorders in Pakistan, which can be achieved by developing local facilities, neonatal screening programmes and collaboration with other countries who are actively working in this field (JPMA 59:815; 2009).

Introduction

Inborn errors of metabolism (IME) results from gene mutations, leading to alteration of primary protein structure and their functions culminating in varied clinical presentations. IEM is a significant cause of morbidity and mortality among young children.¹

There are more than 500 recognized IEM. These are divided into 4 groups.

Group 1 — Aminoacidopathies: Neurologic distress "Intoxication" Type with ketosis. It includes Maple syrup urine disease (MSUD).

Group 2 — Organic acidopathies: Neurologic distress "intoxication" type with ketoacidosis and hyperammonemia. It includes Methylmalonic academia (MMA), Propionic academia (PA), and Isovaleric academia (IVA):

Group 3 — Primary Lactic Acidosis (PDH PC ETC def.) With Neurologic Distress: "Energy Deficiency" type. Enzyme defects in Krebs's cycle leads to respiratory chain

deficiencies. The most common of these disorders are pyruvate dehydrogenase (PDH), pyruvate carboxylase (PC) and electron transport chain (ETC) deficiencies.

Group 4a — Urea Cycle Disorders (UCD): Neurologic Distress due to "Intoxication"; Hyperammonemia without Ketoacidosis.

Group 4b — Nonketotic hyperglycinemia (NKH): Neurologic distress due to "energy deficiency"; type without ketoacidosis and without hyperammonemia.

Group 4c — Lysosomal Storage disorders Without Metabolic Disturbances. These disorders are GM 1 ganglioidosis, Gaucher's disease, Niemann-Pick disease type C, MPS V11 and Sialiodosis. Others are glycogenosis (GSD) and gluconeogenesis defects, fatty acid oxidation defects etc.²

The field of IEM is currently experiencing a wealth of new opportunities with regard to both novel therapeutic options and technology for early disease detection.³

Recently neonatal mass screening for organic acidemias, fatty acid disorders and other such similar

metabolic disorders is being seriously considered in several places throughout the world and tandem mass spectrometry or gas chromatography mass spectrometry (MS/GC) are being used.⁴⁻⁶

IEMs can present in the newborn age in a variety of ways. Typically, an IEM is suspected as a result of a suggestive combination of acute clinical symptoms without prior warning. However sometimes with non-specific clues like unexplained neonatal death, or presence of a previously affected child.⁷

Patients with such disorders can have acute symptoms such as lethargy, hypotonia, tachypnea, convulsions, vomiting or may present with developmental delay or mental retardation and could die of 'obscure causes' despite the fact that a number of such patients could potentially achieve normal growth and development if early detection and intervention were feasible.⁸

There is a great dearth of local data on the subject as there is a lack of investigation facilities to make the diagnosis. There is always a suspicion that because of the higher consanguinity among the community these disorders might occur with a higher prevalence. Therefore we undertook this study in collaboration with IEM selective screening at Japan to see the prevalence of these errors in our setup.

Children in age group birth to ten years of age with high index of suspicion were included in the study with the following criteria. Positive family history of similar illness or death which remained undiagnosed. Children with history recurrent episodes of severe or persistent vomitings for which no infective or surgical cause was found. History of undiagnosed neurological symptoms like coma, convulsions or developmental delay was included. History of respiratory distress with metabolic acidosis was included while those with infective etiology, documented septicaemia and those with clinical and radiological findings of chest infections were excluded.

Methods

This cross-sectional comparative study was conducted at National Institute of Child Health Karachi in collaboration with IEM Selective Screening Unit at Sapporo City Institute of public health Japan.

Study was conducted for period of 3 years from January 2004 to December 2006. Sixty-two children with clinical suspicion of inborn errors of metabolism were evaluated. Biochemical investigations included were blood gases analysis, serum ammonia levels in all patients and if indicated serum blood sugars, ketones and lactic acid levels.

If laboratory findings were significant than dried blood samples (DBS) and dried urine samples (DUS) were sent to

IEM screening unit at Japan to establish definitive diagnosis.

Dried blood sample specimen collection is relatively simple and is done by collecting few drops of blood of about 3mm in diameter on specialized filter paper (Guthrie card) of size 12cm x 8cm which was provided by the screening institute at Japan.

Dried Blood analysis Included: Amino acid Analysis (Methylmalonic aciduria, Propionic acidemia, Multiple carboxylase deficiency, Dicarboxylic aciduria, Tyrosinemia, Alcaptonuria, Uracil, Orotic aciduria, Maple syrup urine disease, Isovaleric acidemia, 3M-crotonylglycinuria, 3H-3M-glutaric aciduria, β -ketothiolase deficiency, Hyperoxalic aciduria, pyroglutamic aciduria, Lactic acidemia); Lactic and pyruvic acids; Galactose levels (in neonates and infants); Acylcarnitine by Tandem Mass; mtDNA MELAS/MERRF mutation; Biotinidase and Total cysteine /homocysteine.

Dried urine samples were collected on another sheet of filter paper size 12cmx 8cm by soaking the paper with the freshly voided urine of patient. Samples were left to dry for 3hrs and within 24hrs were mailed via regular post to metabolic screening laboratory for analysis by HPLC (High Performance Liquid Chromatography) and Tandem mass spectrometry.

Dried Urine Analysis Included: Amino acid Analysis, Organic acid Analysis, Orotic acid and Uracil, Total glycosaminoglycan and Total cysteine /homocysteine.

Results

Inborn errors of metabolism were suspected in 62 children, 16 children (9 boys and 7 girls) were found to have positive results and 46 children had normal results. Mean age of presentation was 11.7 ± 20 months. Male to female ratio was 1.2: 1($p=0.716$).

We looked for the association of certain host factors in children with IEM positive results .Among the host factors, we did not find our results to be statistically significant for sex of the child ($p=0.716$), history of consanguinity in family ($p=0.281$) or any relevance with the

Table-1: Association of Host factors in Children with IEM Positive Results.

Total no children (n=62)	IEM positive (n=16)	P value
Sex(M:F) 39:23	9:7	0.716
Consanguinity(40)	13(32.5%)	0.281
Death of siblings(20)	14(70%)	*0.027
Age		
<1month (14)	2 (14.2%)	
1-12months (36)	10 (27.7%)	0.539
1-5yrs (7)	3(42%)	
5-10yrs (5)	1 (20%)	

Note: * P-value < 0.05 considered to be statistically significant.

age of the child ($p=0.539$) though majority of the children with positive results were less than 1 year of age, but we found our results to be significant for history of death of the siblings in the family ($p=0.027$) (Table-1).

Table-2 shows comparison of clinical features of

Table-2: Comparison of clinical features of IEM positive versus normal group.

Total (n=62)	IEM positive (n=16)	Normal (n=46)	P value
Clinical features			
Vomiting (29)	8(27.5%)	21(72.4%)	0.978
Convulsions (32)	9(28.1%)	23(71.8%)	0.537
Resp. distress (33)	13(39.3%)	20(60.6%)	*0.042
Develop. Delay (21)	10(47.6%)	11(52.3%)	*0.048

Note: P-value < 0.05 consider to be statistically significant.

children with the positive results versus those with the normal results. Among the clinical presentations we found significant association of respiratory distress ($p=0.042$) and developmental delay ($p=0.048$) in children with the positive results and not significant for vomiting ($p=0.978$) and convulsions ($p=0.537$).

Similarly in biochemical labs results 14 /16 children with positive results had raised serum ammonia levels ($p=0.027$) and 13/16 had metabolic acidosis ($p=0.007$) who presented with respiratory distress and insignificant for other metabolic labs, 4/16 had raised Lactic Acid levels ($p=0.839$), 2/16 had ketosis ($p=0.839$) and 1/16 had hypoglycaemia ($p=0.086$).

Out of 16 children with the positive results, 10 (62.5%) showed organic acidemias which includes propionic acidemia (3) biotinidase deficiency (2) multiple carboxylase deficiency (1) cobalamin deficiency (1) was identified on the basis of high levels of propionylcarnitine levels but for definitive diagnosis by complement studies in fibroblast has been requested from the screening unit at Japan, but we have not yet received the report. Methylmelonic acidemia (1) β -ketothiolase deficiency (1) and Isovaleric acidemia. (1). One (6.2%) had Urea cycle defect- Ornithine Transcarbamylase (OTC) deficiency which was characterized by raised Orotic aciduria in dried urine sample differentiating it from carbamylphosphate synthetase deficiency (CPS). Five (31.2%) were found to be positive for congenital lactic acidemias.

Collecting accurate data on the frequency of metabolic disorders is difficult, because the information collected is representative of the particular area or the population group. Table-3 shows the breakup of positive results with their reported incidences elsewhere in the world.

Among children with positive results, of 10 patients

Table-3: Breakup of IEM (positive results), 16/62.

Type of IEM	NICH Positive cases (n=16)	Incidence reported elsewhere in worldwide
Organic acidemia	10	
Propionic acidemia	3	1/ 100,000 live birth in US
Biotinidase deficiency	2	1/137,401 in US
Multiple carboxylase deficiency	1	<1 in 100,000 babies in US
Methylmalonic acidemia	1	1/ 48.000 in US
Cobalamin deficiency	1	3-16% in US population
?-Ketothiolase deficiency	1	50-60 all over world
Isovaleric acidemia	1	1/ 230,000 in US
Congenital Lactic acidemia	5	250 new cases/year in US
Phenylketonuria (PKU)	0	1 /15,000 in US
Urea cycle defect	1	1/ 30,000 in US
Galactosemia	0	1 /40- 60,000 in US

NICH: National Institute of Child Health Karachi.

with organic acidemias, 5 expired (1-Methylmelonic acidemia, 2-propionic acidemias, 1-Biotinidase deficiency, and 1- β -ketothiolase deficiency) 1 case of Biotin deficiency was lost to follow up.

Children were initially treated for acute crisis with intravenous fluids, sodium bicarbonate for metabolic acidosis, protein restrictions, carnitine supplements and sodium benzoate for raised ammonia levels. Currently we are treating 4 children with organic acidemias which includes 1 propionic acidemias and 1 isovaleric acidemia with low protein diet 1-1.5gm/kg/day and L-carnitine supplementation (100mg/kg/day). One (1) with biotinidase deficiency has been put on biotin supplementation (10mg/day) and (1) child with cobalamin deficiency has been started with carnitine and cobalamin supplementation (1mg/day) along with the protein restrictions.

Five children who presented with lactic acidemias, four unfortunately did not survive and one child was started on dichloroacetate (25-100mg/kg/day) arranged from Japan, as a supportive treatment to maintain the oxidation of glucose, lactate, pyruvate and blunt the blood lactate levels from rising, although the clinical outcomes are variable. Unfortunately this child was also lost to follow-up, so we do not know the course of illness. One (1) pt with OTC deficiency has been started on sodium benzoate (250-500mg/kg/day) and citrulline supplementation (200-400mg/kg/day) and protein restrictions (1gm/kg/day).

Surviving patients are coming for follow up and their frequency of acute crisis has decreased, but long term follow up is awaited.

Discussion

These are significant number of positive cases that were seen in this series of patients. Practically, similar data in the local population is not available for reference so there is

nothing to suggest the existing state of affair regarding the prevalence or occurrence of these disorders in Pakistan. With the existing state of higher consanguinity among families in our community it was always thought that we would have a higher prevalence of these disorders. This series supports this presumption by identifying high number of IEM cases in the small number that has been looked at. Death of siblings with similar complaints who remained undiagnosed was another outstanding finding that has also been reported by other studies.⁷ This high number of death of siblings in our setup indicates that it is a very significant marker to be alert about the possibility of inborn errors of metabolism and that there is a delay in diagnosis of such diseases in our setup.

Incidence of organic acidemias in our study was 62% most common being propionic acidemias. The reported incidences of these metabolic errors are very rare.^{9,10} Overall, incidence of organic acidemias varies from 3.9-27% reported from different areas of the world belonging to different ethnic groups.¹¹ Incidence or prevalence of congenital lactic acidemias are unknown, however at least 1000 cases exist in United States population.¹²

Interestingly, we have identified a 6-year-old female child with OTC deficiency Urea cycle defect. OTC is the most common UCD inherited as an X-linked trait.¹³ The phenotype is extremely variable. The hemizygote males are more severely affected than the heterozygote females, having mild disease characterized by episodic clinical manifestations due to hyperammonemia separated by periods of wellness and majority (75%) are asymptomatic.¹⁴ Children with these defects have a spectrum of clinical presentation, being a critically ill neonate to that of a previously healthy female with postpartum hyperammonemia.

So far, we have not identified any case of galactosemia or fatty acid defects.

Majority of our children were less than 1 year, this being a common age of presentation of IEM as reported in other studies.² Interestingly four of our children with positive errors were older than one year and have presented late, indicating that a strong suspicion should be kept in mind to screen out such rare defects, even in older children.

The incidence and range of IEM as a cause of developmental delay is likely to vary between different populations, the most recent reported series are from the USA.^{15,16} Unexplained neurological symptoms should always be considered as a feature of IEM.

Respiratory distress was another clinical feature for which we found our results to be significant. It should be taken as an important pointer and strong clinical suspicion should be kept in mind because non-specific symptoms such as breathlessness, vomiting, reluctance to feed, failure to thrive cannot distinguish organic acidemias from wide variety of

metabolic and surgical emergencies. Although acidosis is hallmark of organic acidemias, an uncommon biochemical presentation is hyperammonemia with significant acidosis¹⁷ as is seen in our group of children.

Screening tests like blood gas analysis, estimation of lactate, pyruvate and ammonia levels, urine examination for ketones and neuroimaging provide valuable clues to the underlying metabolic disease.

The early detection of disorders either in the pre-symptomatic or early symptomatic phase should, with early treatment result in prevention of severe illness and long term complications.^{18,19} Furthermore, genetic advice can be offered to families, with the prospect of prenatal diagnosis for the future pregnancies.

Recent technological advances have led to an expansion in inborn errors that can be detected in the newborn periods and in childhood. Tandem mass spectrometry provides a robust method of screening which can detect a number of inborn errors from few drops of blood on filter paper on single analysis.^{20,21}

Significant cost of tandem mass spectrometry screening however has prevented countries like Pakistan from adopting it as a part of their standard screening process. Hence, such outcome data are difficult to obtain for diseases like IEM; this will require intensive collaboration efforts in future.

Limitations of the study include: Congenital lactic acidosis is a presentation of number of inborn errors of metabolism identified on the basis of levels mentioned in materials and methods, but unfortunately further subtypes like mitochondrial disorders could not be diagnosed due to lack of resources. Also, for the definitive diagnosis of Cobalamin deficiency complement studies in fibroblast been have requested from screening unit, Japan.

Conclusion

We have found a significant number of positive cases in our series of patients, establishing the fact that IEM is quite prevalent in our population, though remain under diagnosed. Early diagnosis and treatment may be able to save a number of these children hence there is a need to develop local facilities to establish the diagnosis. Further, studies are needed on our side in future to determine the incidence of rare metabolic disorders in our part of the world, which can be achieved by developing neonatal screening programmes and screening of suspected cases in collaboration with other countries who are actively working in this field.

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