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ORIGINAL ARTICLE

PREVALENCE OF AMINOACIDURIAS IN A TERTIARY CARE PEDIATRIC MEDICAL COLLEGE HOSPITAL

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ABSTRACT: BACKGROUND: Inborn errors of metabolism (IEM) comprises of a diverse group of heterogeneous disorders manifesting in paediatric population. Cases of Inborn errors of metabolism, individually are rare but collectively are common. The timing of presentation depends on significant accumulation of toxic metabolites or on the deficiency of substrate. These disorders manifest by subtle neurologic or psychiatric features often go undiagnosed until adulthood. **OBJECTIVES:** The objectives of the present study was to carry out preliminary screening on urine samples from pediatric population with either metabolic or neurological manifestations for inborn errors of metabolism and to know the prevalence of aminoaciduria in tertiary care setup for early diagnosis and detection. **METHODS:** The present study is a cross sectional time bound study carried out at Niloufer Institute of Child Health, Osmania Medical College, Hyderabad, from August 2013 to July 2014. A total of 119 samples were analyzed from suspected cases of IEM. Samples were analyzed for all physical and chemical parameters and positive cases reported by these investigations were referred for confirmation by TMS, HPLC, and GCMS. **RESULTS:** Among 119 children analyzed, 29 were given presumptive diagnosis of IEM based on screening tests, urinary aminoacidogram by TLC and clinical correlation. Analysis of the data showed that maximum were in the neonatal age group. Out of these 29 positive cases, 17 were generalized aminoacidurias, 2 were branched chain aminoaciduria, another 2 were methylmalonic aciduria, and one cases each of other aminoacidurias. **CONCLUSION:** Early recognition of IEM by screening tests in combination with strong clinical suspicion will help the clinicians to initiate prompt early treatment to prevent lethal neurological complications and developmental delay. These simple screening tests are highly cost effective and will reduce the economic burden of the patients.

KEYWORDS: Aminoacidurias, Prevalence, Tertiarycare pediatric hospital.

INTRODUCTION: The metabolic errors are caused due to lack or deficiency of key enzyme or coenzyme of an intermediary metabolic pathway causing urea cycle defects, aminoacidopathies, Organic acidemias, fatty acid oxidation defects and errors in energy metabolism. The most common clinical presentation associated with IEM includes CNS manifestations, lethargy, poor feeding, recurrent vomiting and failure to thrive. Acute encephalopathy due to hyperammonemia, is observed in urea cycle defects and many organic acidemias.[1] Among the inborn errors, the largest group typically associated with overwhelming metabolic acidosis in infancy is the

Group of organic acidemias, like methylmalonic acidemia, propionic acidemia and isovaleric acidemia. Effects are due to toxic accumulations of the substrates before block, intermediates from alternative metabolic pathways, defects in energy production and use caused by a deficiency of products beyond the block or a combination of these metabolic deviations.[2,3] Age for presentation of clinical symptoms varies for individual IEM and variant forms within the IEM.

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The timing of presentation depends on significant accumulation of toxic metabolites or on the deficiency of substrate.[4-6] Primary aminoaciduria is due to the enzyme defect in the metabolism of aminoacids eg.,Phenylketonuria, Tyrosinemia and Secondary aminoaciduria is due to the defect in the aminoacid transporter in the kidney and intestine e.g. Cystinuria, Hartnup's disease. Newborn screening in Qatar and Saudi Arabia has confirmed that some IEM are more prevalent in the Arab world like homo cystinuria, organic aciduria, and maple syrup urine disease.[7] In view of high incidence of consanguinity in Sudan, IEM are widely spread among ethnic groups.[8] Sudan is one of the first Arab countries to document on existence of IEM, with phenylketonuria reported in 1964 and galactosemia in 1965 by Hassan.[9,10]

MATERIAL & METHODS: The present study is a cross sectional time bound study carried out from August 2013 to July 2014. A total of 119 samples were analyzed from suspected cases of IEM. A voluntary consent to allow the child for participation in the study has been taken. The study has approved by institutional Ethics Committee. Screening tests were considered in patients who presented with any of the following features like convulsions, failure to thrive, regression of milestones, recurrent vomiting, mental retardation, skeletal deformities, organomegaly, metabolic acidosis, hypoglycemia or jaundice.[7,8] In many of the cases family history and sibling history were much contributory for diagnosis, like history of consanguinity, sibling deaths due to similar illness, repeated abortions etc. 25 ml of random sample of urine was collected in a sterile plastic container with 5 drops of 6N HCl as preservative. Sample was centrifuged at 5000 rpm for 15 minutes and supernatant was analyzed for all physical and chemical parameters. Following laboratory investigations were included in the screening protocol - dicts test for reducing substances, Ferric chloride test for Phenylketonuria, Dintrophenylhydrazine test for alpha keto acids, Nitrosonaphthol test for tyrosine, Para nitroaniline test for methylmalonic aciduria, Cyanide nitroprusside test for cysteine and homocysyteine, Ammoniacal silver nitrate test for homocysteine, Thin layer chromatography for amino acids.

RESULTS: During the course of our study, we have analyzed 119 samples, among which 29 were given presumptive diagnosis of IEM based on screening tests, urinary aminoacidogram by TLC and clinical correlation. Table-1 shows the age and sex wise distribution of suspected IEM cases. There was preponderance of males (56.31%) compared to females (43.69%) in the study group. Age distribution showed that maximum participants were between one to six months of age (37.81%) and only 4.2% cases were >5 years of age, indicating that late onset disorders are less prevalent compared to acute conditions manifesting in newborns.

	Total number of samples	**119**
Sex	Males	67 (56.31%)
	Females	52(43.69%)
Age	Neonates	12(10.08%)
	1 month to 6 months	45(37.81%)
	6 months to 1 year	26(21.84%)
	1 year to 5 years	31(26.05%)
	>5 years	05(04.20%)

Table 1: Age and Sex wise distribution of participants

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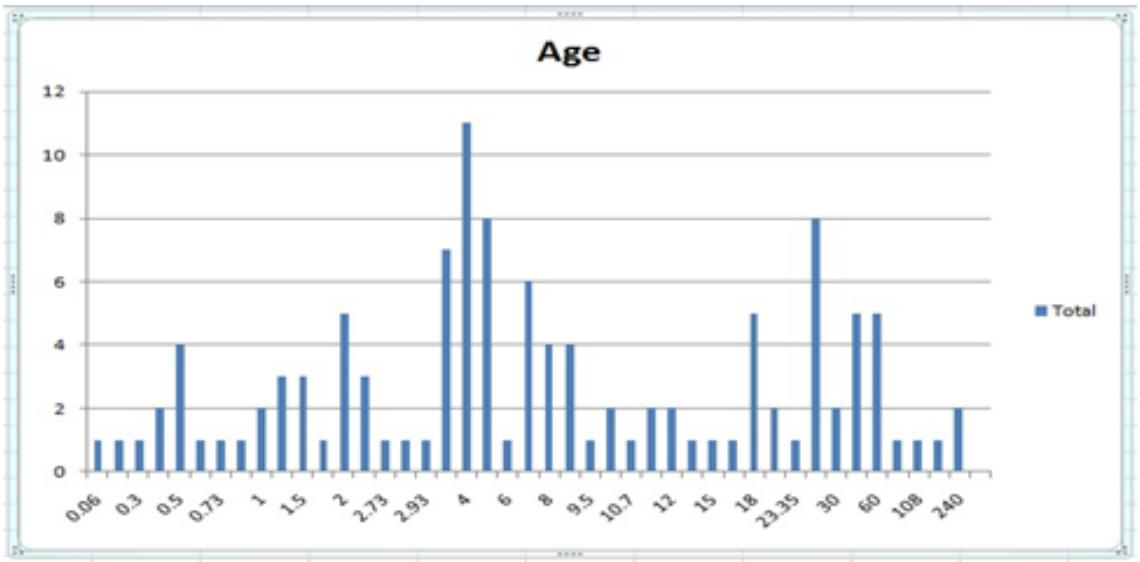
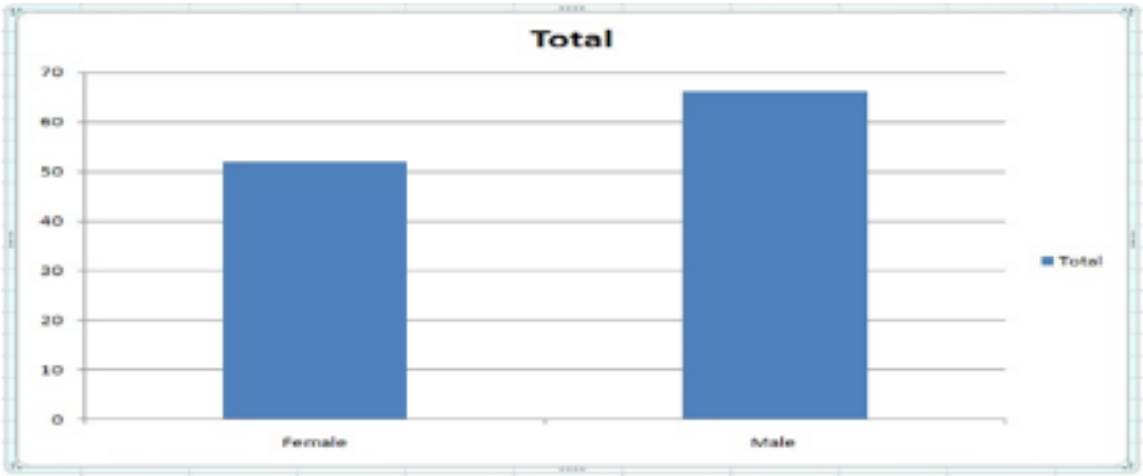


Graph 1



Graph 2: Sex-Distribution

Presentations	No. of Patients
H/O Consanguinity	21(17.64%)
H/O Sibling deaths	10(08.40%)
Convulsions	12(10.08%)
Delayed milestones	10(08.40%)
Failure to thrive	15(12.60%)
Lethargy	12(10.08%)
Vomiting	12(10.80%)
Coarse facial features	05(04.20%)
Encephalopathy	05(04.20%)
Microcephaly	05(04.20%)

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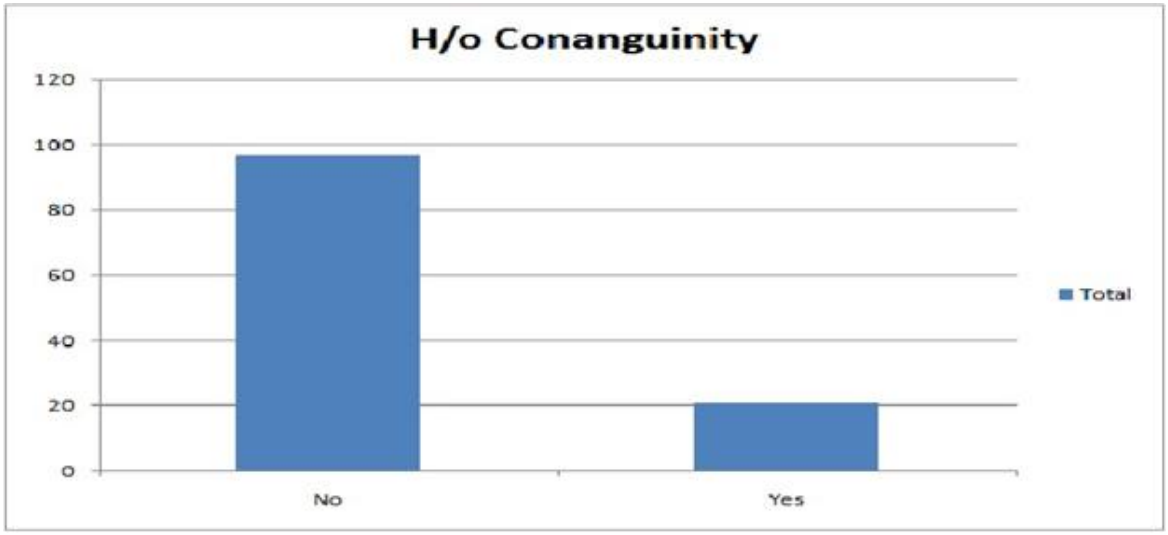
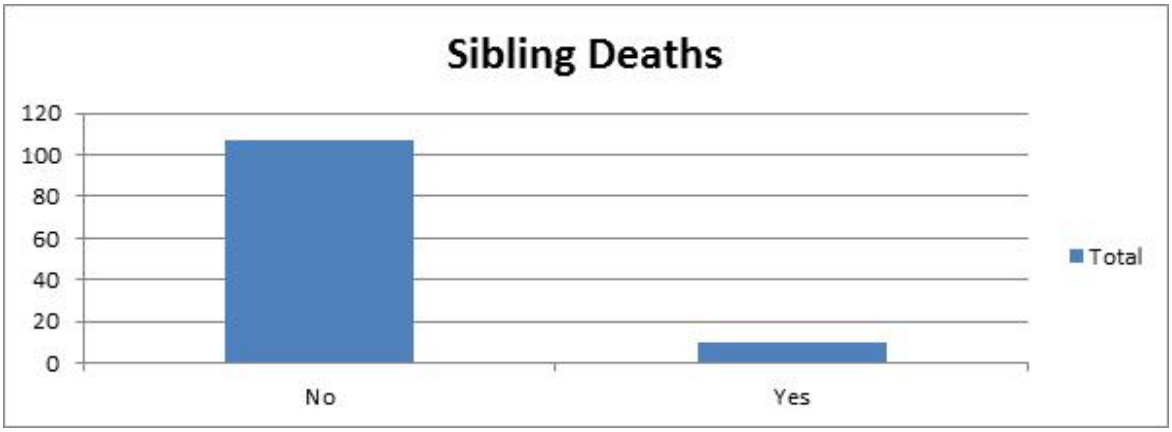
Mental retardation	04(03.36%)
Skeletal deformities	04(03.36%)
Cerebral palsy	02(01.68%)
Corneal deposits	02(01.68%)

Table 2 : Preponderance of clinical presentations among all participants



Graph 3



Graph 4

Metabolic acidosis	24(20.16%)
Hypoglycemia	10(08.40%)
Organomegaly	10(08.40%)
Hyperbilirubinemia	01(0.84%)

Table 3:Commonest laboratory findings among all participants

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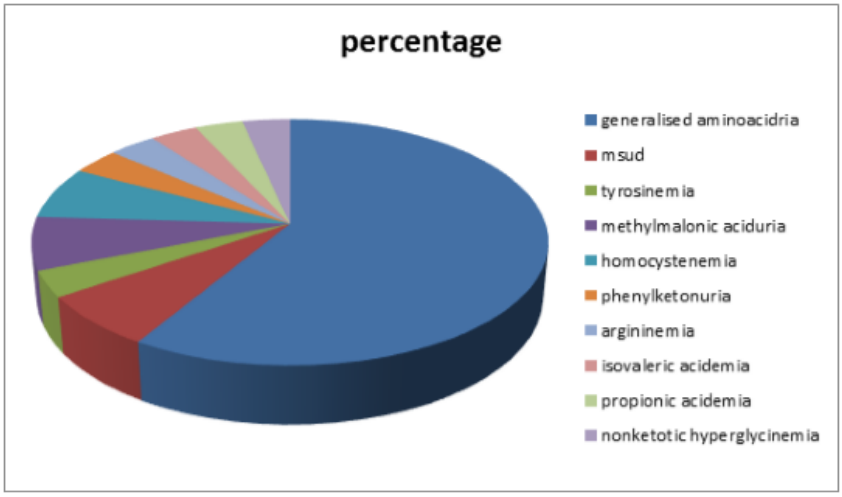
Total number of samples analysed		119
Total number of positive tests reported	29	
Generalised aminoaciduria	17	(58.62%)
Branched chain aminoaciduria(MSUD)	2	(6.89%)
Tyrosinuria	1	(3.44%)
Methylmalonic aciduria	2	(6.89%)
Homocysteinuria	2	(6.89%)
Phenylketonuria	1	(3.44%)
Argininemia	1	(3.44%)
Isovaleric academia	1	(3.44%)
Propionic academia	1	(3.44%)
Non ketotic hyperglycinemia	1	(3.44%)

Table 4: Positive screening tests reported



Graph 5

Analysis of various clinical presentations and available family history is shown in Table-2. The data suggests that the most common presentations were consanguinity (17.64%), failure to thrive (12.60%) and convulsions, lethargy and vomiting (10.08%). Common laboratory features were metabolic acidosis (20.16%) followed by hypoglycemia (8.40%) (Table-3)

Table 4 shows various aminoacidurias identified by screening. Further detailed analysis of the data (Table-5) showed that, among the children who presented with convulsions, 55% showed positive screening tests. Maximum were in the neonatal age group. Out of these 29 positive cases, 17 were generalized aminoacidurias, 1 were branched chain aminoaciduria, and two cases of methylmalonic aciduria, and one cases each of tyrosinuria and phenylketonuria. Among children who presented with developmental delay, >50% showed positive screening tests. Most of them were between 6 months to 3 years. Among these 12 cases, 3 were having generalized aminoaciduria, 1 presented with pheylketonuria and 1 showed branched chain aminoaciduria 1 presented with argininemia.

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	Convulsions	Developmental Delay	Metabolic Acidosis	Hypoglycemia
Total cases	**33**	**25**	**24**	**10**
Positive screening tests	18 (55%)	13(52%)	12(50%)	5(50%)
Age - Neonates	8	0	3	4
- 1month-1 year	6	8	6	0
- 1-5years	2	5	3	1
Sex - Males	9(52.94%)	8(61.53%)	7(58.33%)	3(60%)
-Females	8	5	5	2
Generalised aminoaciduria	8	6	5	1
Branched chain aminoaciduria MSUD	2	2	0	0
Methylmalonic aciduria	2	0	2	1
Tyrosinuria	1	0	1	1
Phenylketonuria	1	1	0	0
Argininemia	0	1	1	
Non ketotic hyperglycinemia	1	0	1	1
Propionic acidemia	0	1	1	0
Isovaleric acidemia	0	0	1	1
Homocysteinuria	2	2	0	0

Table 5: Predominant features of positive screening tests

DISCUSSION: IN most of the aminoacidurias Seizures were a dominant symptom (27%), followed by delayed milestones (21%) as is reported in literature. Various studies have reported that, over one-third of the IEMs are characterized by CNS involvement and neurological symptoms are the most prominent clinical problems associated with them. Routine screening for IEM in children with developmental delay has a diagnostic yield of approximately 1% that can increase to 5% in specific situations such as in the case of relatively homogenous and isolated populations or if there are clinical indicators. Metabolic acidosis was the second common finding among our study subjects (20%). Among them 50% were positive for screening tests.

Two of them were positively diagnosed as cases of methlymalonic aciduria, 2 had branched chain aminoaciduria and one showed tyrosinuria. The group of organic acidemias including methlymalonic, propionic and isovaleric acidemias are among the important causes for wide anion gap metabolic acidosis. Study, maximum cases (58%) were nonspecific generalized aminoacidurias. The predominant aminoacids found in urine were glycine, serine, alanine, glutamate using TLC. In a 30 year survey, Mattingley JM reported that nonspecific generalized aminoaciduria was the most frequent abnormality found, comprising 70% of abnormal results, with cystinelysinuria to be the next most common disorder.[10] Few of these patients may be responsive to vitamin thiamine as reported by Jailkhani. R, Patil Vs.[11] we found 2 cases with branched chain aminoaciduria. Oliviera et al have

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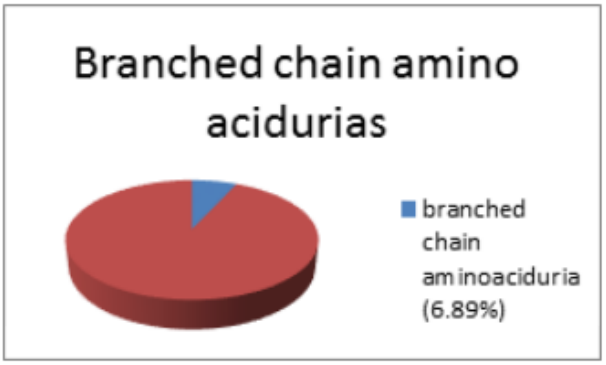
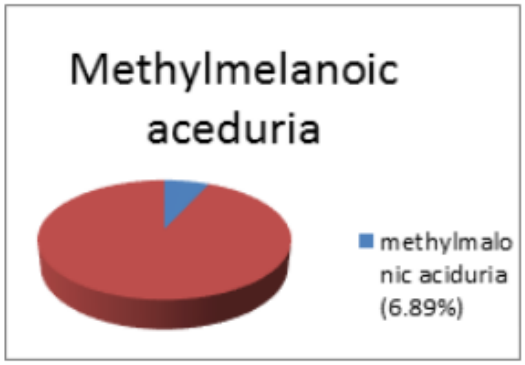
reported highest positivity for tyrosinuria (29%) giving nitrosonaphthol test positive which they attributed to metabolic immaturity.[12]

Most commonly the patients had wide anion gap metabolic acidosis and ketonuria. Dietary therapy has to be initiated within 2 weeks of birth to achieve normal intellect and it should be a long term mode of treatment. We are reporting here 1 cases of transient tyrosinuria who were in the age group of 5 month.[13,14]The child manifested with developmental delay, failure to thrive and convulsions. During the period of our study, we have reported 2 cases of methylmalonic aciduria, which gave strong positive p-nitroaniline test. Songa Y, Lia B, Her Haoa et al, a study from china shows methylmalonic aciduria was the common IEM found and B12 deficiency was the commonest cause.[15] In our patient with PKU, there was microcephaly, muscular hypotonia, cerebral palsy with hypopigmented hairs which are usually associated with classical condition.[16,17]

During the course of our study we have come across with two cases of homocysteinuria with similar complaints in two siblings. Both of them were males aged between 4 and 6 years. Both presented at different times with refractory seizures with mild to moderate developmental delay.[18,19] Later they developed thromboembolic episodes stroke one with right sided and other with left sided hemiparesis. Screening tests done showed positive cyanide levels, later confirmed by TLC method.



Graph 6



Graph 7

CONCLUSION: Screening for IEM in suspected cases should always be done at an earlier age. Positive screening tests give presumptive diagnosis of IEM. Early recognition of IEM by screening tests in combination with strong clinical suspicion will help the clinicians to initiate prompt early treatment to prevent lethal neurological complications and developmental delay. These simple screening tests are highly cost effective and will reduce the economic burden of the patients. Definitive diagnosis by specified tests can be advised for patients positive on screening tests. This will help to overcome the economic constraints in diagnosis of IEM. Hence, there is a need to provide simple screening tests for IEM, as these disorders are not uncommon among those children presenting with nonspecific symptoms.

LIMITATIONS OF THE STUDY: Pre analytical sampling errors due to inappropriate collection & storage of samples might have decreased the positive results. Only few studies are there in Indian context and no proper authentic study material in the Indian context. Ignorance of the population

&lack of motivation to undergo the workup and further studies are needed to prevent deaths and mental retardation.

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