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Spectrum of common and rare small molecule inborn errors of metabolism diagnosed in a tertiary care centre.

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Abstract

Introduction: In most of the disorders, problems arise due to accumulation of substances which are toxic or interfere with normal function, or to the effects of reduced ability to synthesize essential compounds.

Materials and Methods: Total 602 patients were screened in genetic clinic, out of which 112 patients were suspected with IEM. Here we have included data of 40 patients which were diagnosed as small molecule IEM based on TMS/GCMS gold standard and genetic testing in few of them.

Result: Out of 602 patients referred to genetic OPD, 40 patients were diagnosed with small molecule inborn errors of metabolism (6.6%). 112 patients underwent tandem mass spectrometry and urine gas chromatography mass spectrometry.

Discussion: We present the cases of IEM referred to genetic clinic from PICU, NICU, wards and OPD. Most common reason for referral was metabolic encephalopathy, followed by global developmental delay and seizure disorder with less common being hypoglycemia, hepatic failure etc.

Conclusion: By creating this data resource we aimed to leverage an overview of different common and rare IEMs found in our region, and document the genetic variants that are relevant to the diagnosis of IEMs.

Keywords: Metabolism, Mass spectrometry, Gas chromatography, Hypoglycemia.

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Introduction

Inborn errors of metabolism form a large group of genetic diseases involving defects in genes coding for enzymes, receptors, cofactors etc. in metabolic pathways [1]. In most of the disorders, problems arise due to accumulation of substances which are toxic or interfere with normal function, or to the effects of reduced ability to synthesize essential compounds. Inborn errors of metabolism are now often referred to as congenital metabolic diseases or inherited metabolic disorders. Majority of the IEMs are inherited in an autosomal recessive manner.

While individually they are rare, collectively they are common with an overall incidence of greater than 1:1,000 [2]. More than 350 different IEMs have been described to date, and most of these are rare diseases/conditions [3]. IEMs generally lead to encephalopathy, developmental delays, disabilities and even death if left undiagnosed and untreated. It is well documented that extended newborn screening with use of tandem mass spectrometry will prevent irreversible neurological damage and infant mortality [4].

There has been a dramatic increase in understanding, novel diagnostic tests and treatment of these diseases in developed countries. Though Indians have also started utilizing Tandem Mass Spectrometry (TMS) as screening investigation for a suspected IEM or high risk newborn with symptoms, utility of this cost effective technique is still not widespread.

Using relatively simple tests involving the detection of amino acids and acylcarnitines in dried blood spots on filter paper, Tandem Mass Spectrometry (TMS) allows for rapid screening and diagnosis of more than 40 metabolic disorders in amino acids, organic acids, and fatty acid oxidation, substantially improving the efficiency and accuracy of early diagnosis [5,6]. Further confirmation by urine Gas Chromatography And Mass Spectrometry (GCMS) helps in immediate therapeutic intervention and prevention of further morbidity [7]. Genetic testing has further helped to confirm the diagnosis and to opt prenatal testing in future pregnancies.

The present study is the comprehensive data analysis of tandem mass spectrometry and urine metabolic pattern for the diagnosis of IEM by GC/MS in samples received for high-risk IEM screening. Some are diagnosed based on genetic testing.

The current study also revealed that our region has common as well as very rare IEMs being prevalent. Also by creating this data resource we aimed to leverage an overview of different common and rare IEMs found in our region, and document the genetic variants that are relevant to the diagnosis of IEMs.

Materials and Methods

Total 602 patients were screened in genetic clinic, out of which 112 patients were suspected with IEM. Here we have included data of 40 patients which were diagnosed as small molecule IEM based on TMS/GCMS gold standard and genetic testing in few of them. This prospective descriptive study was conducted in MGM medical college in Aurangabad between October 2019 and September 2021. Children between Newborn to 18 years of age admitted in Ward, NICU and PICU with metabolic emergency and diagnosed to have IEM were included in the study.

We included children diagnosed for the first time as IEM during the hospital stay and those who were diagnosed earlier either during newborn period or in the genetic clinic. Diagnosis of IEM was based on biochemical, molecular analysis and/or MRI brain findings. Biochemical testing done to confirm IEM include Tandem Mass Spectrometry (TMS) for detecting abnormality in acylcarnitine profile and amino acid profile, and urinary Gas Chromatography-Mass Spectrometry (GC-MS) for detecting abnormality inorganic acids.

Molecular analysis was done by utilizing next generation sequencing based DNA testing and further validated by Sanger Sequencing. Other tests which were utilized for diagnosis are plasma amino acids, urine or CSF amino acids, urine pterin assay etc. Data were collected by history taking, examination and primary metabolic workup followed by biochemical and molecular testing. Clinical parameters collected include the age of presentation as a crisis, newly diagnosed or known entity, and age of initial diagnosis, sex, consanguinity, clinical signs, and symptoms during the presentation, biochemical, molecular and MRI brain findings, diagnosis, course in the ward in terms of death, or discharge.

Details on biochemical tests done and the molecular analysis carried on these patients were studied. We included molecular testing done either immediately after the diagnosis or later from stored DNA. We also got carrier status of parents done when pro-band sample was not available for molecular diagnosis. IEMs were categorized as protein, lipid or carbohydrate metabolic disorder, vitamin responsive disorders. Institutional Ethics Committee approval was obtained. Categorical data were expressed as number and percentage.

Results

Out of 602 patients referred to genetic OPD, 40 patients were diagnosed with small molecule inborn errors of metabolism (6.6%). 112 patients underwent tandem mass spectrometry and urine gas chromatography mass spectrometry, out of which 32 patients were diagnosed with some IEM based on TMS, urine GCMS and/or genetic testing.

8 patients were diagnosed on the basis of MRI brain and/or Genetic testing. Total 40 patients were diagnosed with IEMs. 35 patients (87.5%) were below 2 years of age, and 5 patients (12.5%) were more than 2 years of age (Table 1).

Demographic characteristics	No. of Patients(n)	Percentage (%)
Age		
<2 year	35	87.5
>2 years	5	12.5
Gender		
Male	28	70
Female	12	30
Consanguinity		
Yes	29	72.5
No	11	27.5
Siblings affected		
Yes	9	22.5
76	31	77.5

Table 1. Demographic characteristics.

28 patients (70%) were males, and 12 patients (30%) were females. Parental consanguinity was seen in 29 patients (72.5%) A positive family history or a previous death of a sibling was seen in 9 patients (22.5%). 21 (52.5%) patients presented with acute encephalopathy, which was followed by seizures as the presenting complaint in 19 (47.5%) patients. Global developmental delay and recurrent vomiting was seen in 9 patients each (22.5%), hypoglycemia in 5 patients (12.5%), hepatic failure in 3 patients (7.5%) (Table 2).

Symptoms/signs	No.of Patients(n)	Percentage (%)
Acute encephalopathy	21	52.5
Global developmental delay	9	22.5
Seizure disorder	19	47.5
Hepatic failure	3	7.5
Hypoglycaemia	5	12.5
Recurrent vomiting	9	22.5

Table 2. Common clinical presentations of IEM.

17 patients (42.5%) were found to have organic acidemias, 4 patients (10%) had fatty acid oxidation defects, 6 patients (15%) had disorder of amino acidopathies, 7 patients (17.5%) had mitochondrial diseases, 3 patients (7.5%) had urea cycle defects. Whereas, carbohydrate metabolism defects, purine metabolic defects and neurotransmitter metabolic defects had 1 patient in each group (2.5%) each (Table 3). The details of the disorders detected are shown in Table 4.

Sr. No	Disease category	No.of patients diagnosed(n)	Percentage (%)
1	Aminoacidopathies	6	15
2	Organic academia	17	42.5
3	Fatty acid oxidation defects	4	10
4	Urea cycle defects	3	7.5
5	Carbohydrate metabolic defects	1	2.5
6	Mitochondrial diseases	7	17.5
7	Neurotransmitter metabolic defects	1	2.5
8	Purine metabolic defects	1	2.5

Table 3. Small molecule IEMs diagnosed.

Case. No.	Age of symptom onset/sex	Presentation	Key biochemical findings(micromoles/lit) (normal values)	Diagnosis	Molecular diagnosis
1	9 months/female	GDD	TMS-Phenylalanine–1763, tyrosine–37.3, Ratio–20.5	Hyperphenylalanemia	
			GCMS-increased 2 hydroxy phenyl lactic acid, 3 phenyl lactic acid		
2	3 years 3 months/male	Distension of abdomen, recurrent infections, hepatosplenomegaly, bicytopenia	GCMS-lysine 3-3456(NMT 998)	Lysin uric protein intolerance	Homozygous likely pathogenic variant in SLC7A7 gene in exon 3 c. 110 dupT
			Lysine 4–2976(NMT 2646)		
3	1 year 3 months/male	Failure to thrive, loose stools, hepatosplenomegaly, vitamin d deficiency, acute liver failure	TMS-proline–314(33-301), methionine–75.6(4.77-46), tyrosine–415(18.9-152), Ratio-0.23,	Tyrosinemia type I	Homozygous likely pathogenic variant in FAH gene in exon 2 c.192 G>T tyrosinemia type I
			GCMS- glutaric-2 12.21(8.44), succinyl acetone-OX-1–13.13().5), phenyllactic-2–12.45(5.79), 4-OH phenylacetic-2–791(12.51)		

4	6 months/male	GDD, hypotonia	TMS- phenylalanine–1896(25-105), phenylalanine/tyrosine ratio–19.07(0.29-4.31)	Hyperphenylalanemia	Homozygous pathogenic variant, in exon 4, c. 200C>T (pThr67Met) in PTS gene, causing Hyperphenylalaninemia, BH4-deficient, A
			GCMS- phenyl pyruvic OX 2 -236.5(0), phenylacetic 1 -3.17(0-0.4), thiodiglycolic – 1.62(0), phenyl lactic 2 – 427(0-4.9), 4OH phenllactic2 – 194.15(0-7)		
			Plasma amino acid-glutamine- 170(246-1182)		
5	3 months/female	GDD, lethargy, metabolic acidosis, ketonuria	TMS- methionine–3.6(4.6-48), acetyl carnitine–2.32(2.49-62.79), butyryl carnitine–0.04(0.06-1.3), propionyl carnitine/acetyl carnitine–0.64(0-0.5)	Hyperhomocystenemia	
			GCMS–methyl citric acid-4–2183 (NMT2014), methyl citric acid-4–24.85(NMT24), methylmalonic acid-2–8594(NMT1216)		
			Sr homocysteine–82(mcmol/L)		
6	15 days/male	Convulsions, encephalopathy, acute liver failure	GCMS- succinyl acetone–163.9(20-100)	Tyrosinemia I	
Organic acidemias					
7	7 months/female	Breathlessness and drowsiness, encephalopathy, ketonuria	TMS- tiglycarnitine(C5:1)-0.24 (>0.14)	2 methyl 3 hydroxy butyric aciduria	
			Malonyl carnitine/Hydroxy butyryl carnitine-C3DC/C4OH-1.98(>0.5)		
			Urine GCMS- 2 methyl 2 hydroxy butyric acid-2>569.3		
			Tiglyglycine-1906.3 (<992.71)		
			3 OH isovaleric acid-2-853.19 (<330)		
			Hydroxybutyric acid 2		
8	1 year 5 months/female	Loose stools, breathlessness, lethargy, unconsciousness, encephalopathy	GCMS-2-hydroxy glutaric acid 3–505(NMT363.42), glutaric acid–82.33(NMT 65.13),	2- hydroxy Glutaric aciduria	
9	1 year 4 months/male	Fever, breathlessness, unconsciousness, encephalopathy	TMS- leucine/isoleucine/hydroxyproline–677(23.93-383), valine–616(31.28-450), free carnitine-6.38(7-109), propionylcarnitine-12.2(0.12-6.65), tiglycarnitine-0.15(0-0.14), glutamic acid-34.4(47-441), acetylcarnitine-0.73(0-0.55)	Propionic aciduria	

			GCMS-2- methyl butyryl glycine- 42.41(NMT 39.53), 3 hydroxy propionic acid-2–1163(NMT 473), 3 methyl glutaconic acid(E) 2 -363(NMT 271), 3 methyl glutaconic acid(Z) 2–253(NMT 101), glutaric acid 2–820(NMT116), methyl citric acid-4-7345(NMT2014),		
10	2 months/male	Unconsciousness, encephalopathy	GCMS- 5-oxoproline-2–26059(NMT 704)	Oxoprolinuria	
11	4 month /male	Metabolic encephalopathy	TMS- increased C8, alanine, decreased free carnitine	Glutaric aciduria II	
12	10 months/female	Fever, vomiting, lethargy, encephalopathy	TMS-propionylcarnitine5.43(0.12-6.65)	Methylmalonic acidaemia	
			GCMS- 3 methyl glutaconic acid (z)-2–108.78(NMT101.54), methylmalonic acid-2–9612.58(NMT1216)		
13	10 years/female	Altered sensorium, decreased oral intake, metabolic encephalopathy	TMS- acyl carnitines–7.61(9-51), total carnitines–14.81(31-123)	Glutaric aciduria II	
			GCMS- more than 4-fold increase in 3 hydroxybutyric acid, ethylmalonic acid, glutaric acid, fumaric acid, malic acid, acetyl glycine, tiglyglycine, glutaconic acid, tyrosine metabolite, 4 hydroxy phenyl lactic acid		
14	13 months/male	Convulsions encephalopathy,	GCMS- 2 OH glutaric 3 –132(0.6-5.9)	Glutaric aciduria type II	
15	5 months/male	Convulsion, altered sensorium, encephalopathy, metabolic acidosis	TMS- glutaryl carnitine –1.01(0-0.35)	Glutaric aciduria type I	
16	15 days/male	Altered sensorium, encephalopathy	TMS- increased lactate, decreased carnitine level	Propionic aciduria	
			GCMS- increased glutaric acid, ethylmalonic acid		
17	2 year 3 months/male	GDD	GCMS- lactate –21.3(2-12), pyruvate –0.96(0.2-2), increased 2 OH butyric acid, 3 OH butyric acid -2, 2 keto 3 methyl valeric acid -2 , adipic 2(C6), C8, 4 OH phenyl lactic, 4 OH phenyl pyruvate	3 methyl gluta conic aciduria type 5	EXOME-homozygous likely pathogenic variant in exon 5 of DNAJC19 gene causative of 3 methylglutaconic acidura type 5
18	2 years/male	Convulsions, altered sensorium, encephalopathy	TMS-negative	Biotin responsive basal ganglia disease	Exome- likely pathogenic homozygous variant in SLC19A3 gene in exon 3, c.595 T>A causing biotin responsive basal ganglia disease
			Urine GCMS- negative		

19	21 days/male	Breathlessness, excessive cry, convulsions, encephalopathy	TMS- increased methyl malonyl carnitine, propionyl carnitine GCMS- increased 3 OH methylglutaconic acid, 3 OH isovaleric acid,	HMG coA lyase deficiency	
20	9 days/female	Refusal to feed and decreased activity	TMS – increased leucine and valine	Maple syrup urine Disease	Exome- both parents' carrier of heterozygous likely pathogenic variant in BCKDHA gene in exon 9 c.1251delC
21	10 days/male	Encephalopathy, metabolic acidosis, ketonuria	TMS-C3-C3/C0 ratio Urine GCMS-increased 3 hydroxy propionate, methyl citrate, and 3 hydroxy isovalerate and significant ketonuria	Propionic acidaemia	
22	3 days/male	Encephalopathy	TMS - C3 - 11.19, C3/C2 RATIO - 0.63, C3/C6 - 7.1	Methylmalonic aciduria	Heterozygous likely pathogenic Variant 1 – MMUT gene on exon 3, c.643G>T, p. Gly215Cys, Variant 2 – MMUT gene on exon 3, c.692dup, p. Tyr231Ter
23	16 months/male	GDD	TMS-leucine/isoleucine/ hydroxyproline-3190(23.9-383.0), valine - 937(31-450), malonylcarnitine-1.15(0-1)	MSUD	EXOME-Homozygous likely pathologic variant in exon 7 c.868 (p. Gly290Arg) in BCKDHA gene causative of MSUD
Urea cycle defects					
24	8 days/male	Convulsion , metabolic encephalopathy	TMS–increased malonyl carnitine, citrulline–2250(93), citrulline/ arginine ratio– 477.1(4), increased glutamine and methionine	Citrullinemia type I	
25	3 years 10 months/ male	Decreased activity, lethargic	TMS - citrulline – 1180 (93), citrulline/arginine - >10% (4%)	Citrullinemia type I	
26	8 days/female	Encephalopathy	TMS – citrulline – 2230(93), glutamine – 3240(1334), methionine – 177(65)	Citrullinemia type I	EXOM – homozygous likely pathogenic variant in ASS1 gene in exon 15, c.1168G>A causative of classic citrullinemia type I
Fatty acid oxidation defects					
27	3 months/male	Distension of abdomen, vomiting, difficulty in breathing	TMS- alanine 78.2(93-1230), methionine 3.83(4.6-48), ornithine-21(25.14-330), argino succinic acid-3.77(0-2), glutamic acid-49.6(69.76-652), malonylcarnitine-2.25(0-0.5) GCMS-3-hydroxyadipic acid-3 -1332(NMT 597), glycerol 3 phosphate-4 – 608(NMT203)	3 OH acyl CO A dehydrogenase deficiency	
28	6 months/female	Motor developmental delay, hypoglycemia, deranged LFT	TMS- 0.62(0.01-0.34) C6 and C8-4.02(0.01-.038)	Medium chain Acyl CoA Dehydrogenase deficiency	

			GCMS-C6-440% (<30%) non hydroxy dicarboxylic acid C8-29.49% (<0.59%), C10-450% (<11.5%), C12-4.22% (<0.5%)		
29	5 months/male	Global developmental delay, seizures, encephalopathy, metabolic acidosis, hyperlactatemia	C16 OH-0.19 (0.00-0.08)	Long chain 3Hydroxy acyl Co A dehydrogenase Deficiency	
			C18OH- 0.84 (0.07-0.15)		
			Ornithine-253.2(23-216)		
			Alanine-637(72-619)		
			UOA-short chain, medium chain fatty acids and long chain fatty acids only up to C12 (dodecanedioic acid) and their corresponding 3 hydroxy dicarboxylic acids.		
30	2 years/male	Recurrent vomiting and Ketotic hypoglycemia	TMS- C16OH- 0.19 (0.00-0.08)	Long chain acyl Co A dehydrogenase deficiency	
			UOA-2-Hydroxy-butyric acid and 3-Hydroxy-butyric acid		
			Adipic acid and 3-Hydroxy-dodecanedioic acid		
			Normal growth hormone, insulin, and cortisol levels		
31	2 months/male	Yellowish discoloration of body, distension of abdomen, decreased activity, hypoglycemia	TMS- free carnitine – 1.34(7-121), acetyl carnitine – 2.08(2.49-62), butyryl carnitine-0.05(0.06-1.3)	Carnitine uptake defect	
32	9 months/Male	Loose stools, difficulty in breathing, hypoglycemia	TMS- free carnitine – 4.06(7-121), acetyl carnitine – 1.3(2.49-62.79), propionyl carnitine – 0.1(0.12-6.65), butyryl carnitine – 0.05(0.06-1.3)	Carnitine uptake defect	
33	7 years/female	Vomiting, loose stools, repeated episode of hypoglycemia	Decreased free carnitine, increased 3 FFA, increased 3 hydroxybutyrate	Carnitine uptake defect	
Mitochondrial disorders					
34	2 years/male	GDD, convulsions, encephalopathy	TMS- negative	Mitochondria disease	MRI brain – T2 weighted hyperintensities in B/L central tegmental tracts in dorsal pons s/o mitochondrial disease
35	2 years/female	Motor developmental delay, vision loss, fundus shows optic atrophy	TMS-negative	Mitochondria DNA depletion syndrome – 7	EXOM-compound heterozygous variant TWNK gene
					One heterozygous likely pathogenic variant in exon 1, c.1003 C>A and a second VUS in exon 5- c. 2050 A>C causing mitochondrial DNA depletion syndrome 7
36	3 years/male	Convulsions, altered sensorium,	TMS-negative	Mitochondrial encephalopathy with lactic acidosis	MRI – T2 weight images shows B/L

		encephalopathy, breathlessness			hyperintensities in bilateral, basal, ganglia, midbrain, pons and cerebellum, lactate peak on MRS mitochondrial encephalopathy
37	18 months/male	Convulsions, altered sensorium, encephalopathy, neuro regression	Sr. lactate – 2.6	Leigh disease	MRI brain – symmetrical T2 weighted hyperintensities in bilateral, basal, ganglia, midbrain, pons and cerebellum, lactate peak on MRS
Neurotransmitter metabolic defects					
38	5 months/female	GDD with hypotonia	GCMS-2 deoxy tetronic acid-3 – 3688.36(NMT 806), 4 hydroxy butyric acid-2 – 11959(NMT 442), adipic acid-2 – 401(NMT 296), glutaric acid-2 – 589(NMT116), glycolic acid-2 – 1348(NMT 1238)	Succinic semialdehyde dehydrogenase deficiency	EXOME – homozygous likely pathogenic variant in ALDH 5A1 exome 4 c. 701 C>T succinic semialdehyde dehydrogenase deficiency
Disorder of purine metabolic defect					
39	17 months/male	GDD, convulsions, encephalopathy, microcephaly, hypotonia	TMS- negative Urine GCMS- negative MRI- diffusion restriction in posterior limb of internal capsule	Inosine triphosphate phosphohydrolase deficiency	EXOM- ITPA gene detected in homozygous likely pathogenic variant in exon 3 c.137delA (p. Gln46Argfs Ter43) causative of inosine triphosphate phosphohydrolase deficiency (pathogenic variant) AR
Disorders of carbohydrate metabolic defect					
40	4 months/female	Yellowish discoloration of body, distension of abdomen, clay color stools	TMS-arginine – 67(0.78-60)	Classical galactosemia	compound heterozygous variant in GALT gene variant 1- likely pathogenic c.142 C>T in exon 2,
			GCMS-galactitol-6-1827(NMT 788), glycerol 3 phosphate-310(NMT 203), xanthine – 1412(NMT 323)		Variant 2- pathogenic c. 610 C>T in exon 7 causative of classical galactosemia
			Time resolved flouro immunoassay – T GAL – 30(NMT 25)		

Table 4. Disease spectrum of different types of Inborn Errors of metabolism.

Discussion

We present the cases of IEM referred to genetic clinic from PICU, NICU, wards and OPD. Most common reason for referral was metabolic encephalopathy, followed by global developmental delay and seizure disorder with less common being hypoglycemia, hepatic failure etc. IEM could potentially be under-diagnosed and high index of suspicion and team effort is essential to diagnose IEM. Availability of advanced biochemical testing helped in the definitive diagnosis. The article on testing modalities of IEMs has really helped us to reach the diagnoses [8]. Metabolite pattern recognition in all the tests helps to arrive at a specific diagnosis. TMS, GCMS, and HPLC of amino acids in blood and urine are the most common diagnostic modality to aid in definitive diagnosis [9,10].

The most common IEM group in our study was organic acidemias, accounting for 42% of the total IEM. Aminoacidopathies, organic acidemia, and Urea Cycle Disorders (UCD) are known to present as metabolic encephalopathy and hence metabolic encephalopathy was the most common symptom in our study. As seen in study by Kamate et al., maximum number of cases was organic acidemias [11]. In our study, 32 patients (87.5% patients) were less than 2 years, 6 out of them were neonates (18.75%). In study by, Sivaraman et al. [12] almost half have been diagnosed in the neonatal period itself, which is most likely to indicate a more severe spectrum of IEM. This could be due to

early suspicion of team of NICU in this study. Amongst organic acidemias, glutaric academia II (2), propionic academia (2), methylmalonic acidemia (2), glutaric acidemia (1), MSUD (1), Biotin responsive basal ganglia disease (1) were the common organic acidemias which can be picked up on extended newborn screening. A pilot study in India also identified these as common organic acidemias in newborn screening [13].

Amongst the rare organic acidemias diagnosed based on TMS as a screening and GCMS as a gold standard test and genetic study in few, were 2 methyl 3 hydroxy butyric aciduria, 2-hydroxy Glutaric aciduria, Oxoprolinuria, 3 methyl glutaconic aciduria type 5, HMG coA lyase deficiency and riboflavin deficiency etc. L-2 hydroxy glutaric aciduria has been reported in India by Kamte et al. [14] and Balaji et al. [15]. Oxoprolinuria has been reported in by Bhaskaranand et al. [16] in a pediatric patient and an adult patient by Senthilkumaran et al. [17]. A 7-month old girl child with metabolic encephalopathy was diagnosed with 2 methyl 3 hydroxy butyric aciduria which is an X linked dominant rare IEM and not reported in India. 21 days old male child was admitted with metabolic encephalopathy, was diagnosed with HMG CoA lyase deficiency, which is a defect in ketogenesis.

This is not reported in India and has been reported by Sass et al. [18] 2-year-old male child with global developmental delay was diagnosed with 3 methyl glutaconic aciduria type 5 based on homozygous likely pathogenic variants in exon 5 of DNAJC19 gene c.250C>T (pArg84Ter). To date, maximum cases of DCMA reported involve individuals from the Dariusleut Hutterite population, an endogamous population of the Great Plains region of Canada and the northern United States. Update on cases, natural history by Machiraju et al. has described phenotypes in this disease [19]. This patient had global developmental delay with ataxia and micropenis requiring testosterone injections in infancy.

2D echo was normal. Another 2 years old child with global developmental delay, movement disorder and seizure disorder had TMS and GCMS negative but exome sequencing revealed homozygous likely pathogenic variants in SLC19A3 gene in exon 3, c.595 T>A causing biotin responsive basal ganglia disease. There is a case series by Majid et al. [20], Kassem et al. [21] and 3 case reports from India [22-24]. A 9-day old female succumbed to metabolic encephalopathy and TMS was screen positive for MSUD. Carrier screening of parents by next generation sequencing revealed that both parents were carrier of heterozygous likely pathogenic variant in BCKDHA gene in exon 9 c.1251delC.

There is a case series by Bashyam et al. and Narayan et al. from India [25,26]. 3-day old male child succumbed to encephalopathy that also had similar sibling death on day 3 of life, was screen positive for methylmalonic academia. His parents were having compound heterozyous likely pathogenic variants in MMUT gene c.643G>T(pGly215Cys), and second variant c.692dup, pTyr231Ter. Observed variants are already reported in literature. 2 patients with glutaric academia II, 1 patient with glutaric acidemia I, 1 patient with riboflavin deficiency, 1 with 3 methyl glutaconic aciduria types 5 are on regular follow up with dietary modifications and supplements.

Amongst the common aminoacidopathies, 2 patients were diagnosed with tyrosinemia type 1 and 2 with hyper-phenylalaninemia, 1 with hyper-homocystenemia and 1 with a rare amino acid disorder of lysinuric protein intolerance. We have already reported the case of lysinuric protein intolerance [27]. One patient with hyperphenylalaninemia underwent urinary pterin assay and was suspected to be suffering from biopterin pathway defect. His clinical exome analysis revealed homozygous likely pathogenic variant in exon 4, c.200C>T (pThr67Met) in PTS gene, causing Hyperphenylalaninemia, BH4-deficient, A. There is a case series and a case report from India on BH4 deficient hyperphenylalaninemia [28,29].

Amongst urea cycle defects, all 3 patients were diagnosed with citrullinemia, two patients were diagnosed in neonatal age with encephalopathy and third child was diagnosed late with behavioral changes. Citrullinemia was common UCD in latest study from India [30]. Molecular analysis in one patient revealed common mutation of c.1168G> A (pGly390Arg) as described in the study by Bijarniya et al. Amongst the fatty acid oxidation defects, acyl carnitine profile in TMS, was suggestive of 3 patients with carnitine uptake defects, 2 patients with long chain acyl Co A dehydrogenase deficiency (LCHAD), one each with short chain and medium chain acyl Co A dehydrogenase deficiency (SCAD and MCAD). Patients with carnitine uptake defects, MCAD deficiency presented with symptoms of hypoglycemia, hepatomegaly and raised liver enzymes and deranged PT INR.

All patients are under treatment with reduced episode of hypoglycemia following precautionary advice. Patients with SCAD and LCHAD deficiency presented with metabolic encephalopathy with seizure disorder and succumbed to acute encephalopathy. All the FAODs in our study have low prevalence in India with few case reports [31-33]. One patient was diagnosed with neurotransmitter metabolic defect of GABA (gamma amino butyric acid), a girl child with global developmental delay and autistic features. She was diagnosed with c.701 C>T homozygous likely pathogenic variant in ALDH5A1 gene. SSADH deficiency has been reported in India from two studies [34,35]. Approximately 450 cases are diagnosed with SSADH deficiency worldwide [36].

One child was diagnosed with purine metabolic defect. 17 month old child with global developmental delay was diagnosed with inosine triphosphate phosphohydrolase deficiency, based on homozygous pathogenic variants in ITPA gene, c.137delA (pGln46ArgfsTer43). Homozygous or compound heterozygous mutations in ITPA gene are known to cause neurological presentations. One case reported by Karthik et al. [37] has similar features of encephalopathy, global developmental delay and MRI brain abnormalities. Another study by Nicholas has described the similar phenotype [38]. TMS and GCMS was screen positive for galactosemia as in our case of 2 months old girl child who presented with hepatic failure, hypoglycemia and bilateral cataract, her DNA study revealed compound heterozygous pathogenic variants c.142

C>T and c.610 C>T in GALT gene. There are case series and newborn screening studies on galactosemia in India [39-42].

Amongst the 4 patients with mitochondrial disease, all 4 had typical MRI brain findings of symmetrical T2 weighted hyper intensities in bilateral, basal ganglia, midbrain, pons and cerebellum and lactate peak on MRS. A 2-year-old girl with motor delay and optic atrophy underwent molecular testing and compound heterozygous variant in TWNK gene, one heterozygous likely pathogenic variant c.1003 C>A and a second variant of unknown significance c.2050 A>C causing mitochondrial DNA depletion syndrome 7 was detected. All individuals with the IOSCA founder variant in TWNK have been identified in the genetically isolated population of Finland only, where IOSCA is the second-most common inherited ataxia [43]. Other TWNK variants have been described in affected individuals of English, Pakistani, Indian origin [44-46]. In developed countries, newborn screening is being done widely for varying metabolic disorders. The conditions screened are 6, 29, and 23 conditions, in the UK, USA, and Australia, respectively [9]. Among the patients who presented for the first time, more than 50% patients could have been potentially picked up by NBS.

Molecular testing was done in 13 cases (32.5%) of the study cohort. We could not confirm using molecular analysis in a higher proportion and had to do parental carrier screening by clinical exome studies in few. It is a good practice to store DNA even when the testing cannot be done at an acute state during NICU or PICU admission. As all the conditions in the present study are inherited in an autosomal recessive manner, we got 72% (8/11) of a pathogenic variant in homozygous status and only 23% (3/13) as compound heterozygous. In India due to the high prevalence of consanguineous marriage and endogamous population there is a high chance of these conditions occurring in the homozygous state rather than the compound heterozygous state. In the present genomic era, next-generation sequencing is the most common test utilized to detect single gene disorder exome sequencing is an effective technology for diagnosing metabolic disorders. Molecular analysis has not only helped the index child but also in prenatal diagnosis in 3 families and fetuses were carrier for the disease.

As this study describes only the short-term outcome, it is not taking into account the death that could potentially happen outside the study period or which could happen at a different hospital or home. This is one of the limitations of the study. Strengths of our study are a detailed description of biochemical testing aiding the definitive diagnosis and diagnosing rare IEMs and mutations unique to our region.

Conclusion

TMS and urine GCMS is helpful in facilitating early diagnosis and timely treatment of inherited metabolic disorders. Because of high degree of consanguinity and marriages in same community, common as well as many rare inherited metabolic diseases were diagnosed. Clinico-etiological profile study has thrown light on clinical features, natural course of many common and rare IEMs and it may provide clinicians with a deeper understanding of these conditions, allowing for improved early diagnosis and treatment of these diseases. By creating this data resource we aimed to leverage an overview of different common and rare IEMs found in our region, and document the genetic variants that are relevant to the diagnosis of IEMs.

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