Testing Modalities for Inborn Errors of Metabolism – What a Clinician Needs to Know?

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The present century is being hailed as the century for genetic therapies, and inborn errors of metabolism is leading the way. As we gear ourselves for treating children with genetic and metabolic disorders, the key is to recognize them early and accurately for best outcomes. In these changing times with advent of technology, clinicians are now more aware, exposed and well equipped with the armamentarium of diagnostic modalities. However, it is difficult to choose between the tests without a baseline knowledge about testing for genetic and metabolic disorders. The key question for a clinician when dealing with a suspected metabolic disorder case is 'what test to order' and 'how to proceed.' The current article provides a rational view on the various laboratory testing modalities available for diagnosis of inborn errors of metabolism. The article provides details of the basic and advanced metabolic tests that can be ordered in appropriate settings.

Keywords: *Diagnosis, Genetics, Metabolic disorders, Tandem mass spectrometry*.

As we begin to settle down in the 21st century, the realization that technology has made it possible to move its application from the bench to the bedside is dawning. Improvements in neonatal health care have led to an unprecedented decline in infant mortality rate to 33 per thousand live births [1], shifting the focus on to other 'significant' causes of pediatric morbidity and mortality. Inborn errors of metabolism (IEMs), the term coined by Sir Archibald Garrod in 1908 [2], are a group of disorders which affect nearly 1 in 1000 children [3]. The diagnostic ability has preceded the therapeutic advances by a few decades. The recognition of inherited metabolic disorders has increased with availability newborn screening and metabolic testing in both public and private domain. Technological advances in the field of diagnostics has benefitted patients with metabolic disorders and this appears to be appropriate time to focus on the correct technology to use [4,5]. This review will elaborate upon the various testing modalities for screening and confirmative diagnosis of the IEMs.

A common presentation of IEMs is an apparently healthy child presenting acutely with a short history of illness, which is worsening progressively. The symptoms could be as mild as a fever, or as severe as encephalopathy or status epilepticus. Few common clinical presentations are (*i*) acute (and recurrent) episodes of symptoms such as vomiting, respiratory distress, ataxia, lethargy or coma with or without seizures, hypotonia, hypoglycemia or hyperammonemia; (*ii*) chronic and progressive symptoms such as developmental delay, epilepsy or other neurological illness, or (*iii*) organ specific presentation (single or multiple) such as hepatopathy, cardiomyopathy, neuro-muscular, renal, gastroenterology or hematological illness. These children should be evaluated for a metabolic cause particularly with a past history or family history of similar illness in a sibling. Presence of consanguinity makes the suspicion of an IEM stronger. A complete discussion on the various clinical approaches to diagnosis of IEMs is out of scope of this review; the readers are suggested to refer to other resources [6-8].

IEMs can be detected on routine tests such as blood gas, blood glucose, blood ammonia and ketones in urine, which can be followed by more advanced tests. The tests utilized for diagnosing an IEM are detailed below.

Basic Metabolic Tests

Some tests on blood and urine can be performed at the bedside or quickly in a routine laboratory. These tests are helpful in screening for many IEMs, but are not diagnostic by themselves.

Urine Analysis

The urine is an excellent source of crucial metabolites as the excess pathological metabolites in the body are excreted out in urine. Urine should be examined for the following observations before subjecting it to advanced biochemical testing [8-12].

Color: Yellow (light to dark) may indicate bilirubin/drug metabolites, while coffee or cola colored may indicate rhabdomyolysis, and darkening on standing (exposure to light) may be observed in porphyria and alkaptonuria (***Table* I**).

Odor: Sweet odor in ketosis, maple syrup/ burnt sugar in maple syrup urine disease (MSUD) and mousy in phenylketonuria (PKU) have been described. ***Table* I** outlines the odors described in the various IEMs.

Urinalysis: Urinary pH, specific gravity, creatinine concentration, glucose/reducing substances, protein/ albumin/micro-albumin, ketonuria are important preliminary investigations in IEMs.

Blood Tests

Complete blood count with differential counts: Anemia, thrombocytopenia, leucopenia or leucocytosis is noted in organic acidurias. Peripheral blood smear is helpful for the type of anemia, signs of hemolysis, and for abnormal cells. Megaloblastic anemia can point towards a vitamin B_{12}/cobalamin or folate absorptive or intra-cellular utilization disorder.

Blood Glucose, Electrolytes, Lactate, Acid base balance/ arterial blood gas, Ammonia, and Ketosis (Acronym-GELAK): Stringent conditions need to be met with regards to the transport and analysis of both lactate and ammonia. Ammonia estimation should be done from a free flowing blood sample obtained from either arterial or venous puncture. The sample should be collected in a preferably pre-chilled tube containing EDTA (for ammonia) as an anticoagulant. Patient should be fasting (or at least 4-6 hours after feeding) and non-stressed such that it is not collected after a difficult venipuncture. This, however, would not hold true for an acutely sick child when samples should be collected immediately upon arrival or in acutely decompensated state, irrespective of feeding status. Samples should be transported on ice, separated within 15 minutes of collection, and analyzed immediately to prevent artefactual increase in generation of ammonia from RBC degradation or deamination of amino acids by enzymes such as gamma glutamyl transferase. Normal levels of ammonia are <110 µmol/L (neonates), <80 µmol/L (infants), <50 µmol/L (older children) and <35 µmol/L (adults). Lactate analysis can be done from whole blood or plasma collected in a

TABLE I INTERPRETING ABNORMAL COLOR AND ODOUR OF URINE

Characteristic	*Disorder*	*Compound*
Urine Color		
Dark brown or black	Alkaptonuria	Homogentisic acid
	Hemoglobinuria/Myoglobinuria	Hemoglobin/Myoglobin
Red	Hematuria	Erythrocytes
	Porphyrias	Uroporphyrin, Coproporphyrin
	Ingestion of colored foods - beet	Anthrocyanine
	Red dyes	Rhodamine B, Phenolphthalein
Blue	Hartnup disease	Indican
Blue/brown	Alkaptonuria	Homogentisic acid
Urine Odor		
Maple syrup/ burnt sugar	Maple syrup urine disease	Sotolone, 2-oxoisocaproic acid
Sweaty feet	Isovaleric acidemia, Glutaric acidemia type II	Isovaleric acid
Sulfur	Cystinuria	Hydrogen sulfide
Boiled cabbage	Tyrosinemia type I, Methionine malabsorption	2-hydroxybutyric acid, 2-keto-4-methiolbutryric acid
Old fish	Trimethylaminuria, Dimethylglycine	Trimethylamine, dimethylglycine
	dehydrogenase deficiency	
Cat's urine	Multiple carboxylase deficiency,	
	3-methyl crotonyl-CoA carboxylase deficiency	3-Hydroxyisovaleric acid
Mousy	Phenylketonuria	Phenylacetic acid
Maple syrup/ burnt sugar	Maple syrup urine disease	Sotolone, 2-oxoisocaproic acid

sodium fluoride or heparinized vial. Sample should be transported on ice slurry or an ice pack within 30 minutes of collection. Once aliquoted, the plasma is stable on room temperature for 8 hours. Blood collected from artery is ideal but a free flowing venous blood sample is also acceptable. The lactate value on blood gas is reliable if the machine is calibrated. There are two units used frequently for lactate values – mg/dL and mmol/L. Normal range of lactate (both in blood in CSF) are 2-20 mg/dL and 0.2–2.0 mmol/L at all ages.

Liver function tests (LFT): The list of IEMs with liver involvement is exhaustive–ranging from small molecule disorders such as urea cycle, amino acid or organic acid disorders to mitochondriopathies and large molecule disorders such as peroxisomal and lysosomal storage disorders. For more in-depth information on IEMs related to liver disease readers are directed towards other reviews [13,14].

Renal function tests: Serum creatinine indicates renal function derangement, if high; and creatine synthetic or transporter defect, if low.

Uric acid: High levels in presence of features such as intellectual disability and self-mutilation is highly suggestive of Lesch-Nyhan Syndrome (HPRT deficiency – purine recycling disorder). High levels may also suggest disorders of carbohydrate disorders such as glycogenolysis or gluconeogenesis. A low level may point towards xanthine/hypoxanthine disorder or a molybdenum cofactor deficiency (MoCD).

Creatine kinase: It is elevated in metabolic disorders affecting the muscle causing glycogenolysis, or rhabdomyolysis, and in disorders of energy production such as fatty acid oxidation defects, gluconeogenesis defects or a mitochondrial disorder.

Plasma total homocysteine level: This is elevated in disorders of vitamin B_{12} or folate metabolism, and in classic homocystinuria due to deficiency of cystathionine beta-synthase enzyme that utilizes pyridoxine as a co-factor. Low homocysteine level may be indicative of a methionine disorder such as methyl adenosyl transferase deficiency. Normal range for plasma homocysteine is 5-15 µmol/L at all ages.

Lipid profile: This is deranged in glycogen storage disorders, lipoprotein disorders, transient infantile hypertriglyceridemia. Dyslipidemia is also noted in Wolman disease, fructose-1-6-bisphosphatase deficiency and lipid storage disorders such as Niemann-Pick disease.

Prolactin levels: This may be elevated in neurotransmitter disorders (dopamine synthesis). Normal level in serum is 5 to 20 ng/mL (5 to 20 µg/L).

Copper levels (*in plasma*): This may be decreased in Wilson disease, Menkes, aceruloplasminemia, and MEDNIK syndrome; and increased in peroxisomal disorders.

Iron levels: Increased serum iron/ferritin is observed in hemochromatosis and peroxisomal disorders

ADVANCED METABOLIC TESTS

These are biochemical reactions utilized for making a specific diagnosis of a single or group of disorders. These tests measure specific metabolite, enzyme or a cofactor. Specific indications for these tests with classification of IEMs are provided in ***Table* II.** The classification is proposed by the Society for study of inborn errors of metabolism (SSIEM) according to biochemical basis [7].

Enzyme Assay

Testing for activity of an enzyme requires recreating/ simulating the actual enzymatic reaction outside of body using appropriate body fluids/tissue (leukocytes, plasma/ serum, skin fibroblasts or liver biopsy specimens), and using artificial or natural substrates [15].

Lysosomal storage disorders: More than 24 enzymes are known, and can be tested in blood (leucocytes, sometimes plasma) and cultured skin fibroblasts. Examples are enzymes for Gaucher disease, Niemann Pick disease [13]. Usual requirement for testing is 4-5 mL blood in heparin vial (or sometimes EDTA vial). Lysosomal enzymes can also be measured on a dried blood spot [14]

Galactosemia: Galactose-6-phosphate uridyl transferase (GALT), Galactokinase (GALK), and Galactose-1-phosphate uridyl epimerase (GALE) can be performed on red blood cells (RBCs) only; therefore, blood in heparin vacutainers is required. Semi-quantitative testing can also be performed on dried blood spots (Beutler spot test), and is useful in newborn screening.

Biotinidase deficiency: This enzyme assay can be performed in serum (quantitative) or on dried blood spots (semi quantitative), and is useful in newborn screening.

Other small molecule disorders: Enzyme assays for fatty acid oxidation defects (FAOD), gluconeogenesis (hereditary fructose intolerance, fructose 1-6-biphosphatase), glycogen storage disorders (GSD), urea cycle defects or organic acidurias, tyrosinemia, cystinosis, and porphyrias used to be the standard diagnostic modalities until molecular diagnosis became widely available. There are limited accredited laboratories for enzyme assays [16]. Majority of these disorders would

TABLE II METABOLIC TESTS FOR DIAGNOSIS OF IEMS (ACCORDING TO SSIEM CLASSIFICATION) [7]

Disease group/disorder	*Metabolic derangements**	*Basic metabolic tests for diagnosis*	*Diagnostic modalities of choice*
Disorders of amino acid, organic acids and peptide metabolism	Acidosis, hypoglycemia, hepatic and renal dysfunction, cardiomyopathy	GELAK (glucose, electrolytes, lactate, acid-base, ammonia, ketones), homocysteine	Tandem Mass spectrometry (MS/MS), U/HPLC quantitative amino acids, Urine GC-MS, Succinylacetone (Tyrosinemia type 1)
Disorders of carbo-hydrate metabolism	Hypoglycemia, ketosis, acidosis, hepatic and renal dysfunction	Glucose, other sugars, insulin, acid-base, lactate, ketones, LFT	Fasting studies, enzyme assays (Galactosemia), genetic testing
Disorders of fatty acid and ketone body meta-bolism	Hypoglycemia, rhabdo-myolysis, hyperammonemia, cardio-myopathy, liver dysfunction, Reye-like syndrome	Glucose, lactate, ammonia, ketones, acid-base, Creatine kinase, uric acid	MS/MS, Urine GC-MS
Disorders of energy metabolism	Recurrent acidosis, hyper lactatemia, hyperammonemia, liver dysfunction, Myopathy, cardiomyopathy, renal tubular dysfunction	Glucose, lactate, Pyruvate, Kreb cycle metabolites, ketones, organic acids	Lactate/ pyruvate ratios (plasma, CSF), Urine GC-MS, Mitochondrial respiratory chainenzymology, mtDNA and nDNA gene studies
Disorders in the meta-bolism of purines , pyrimidines and	Increased or decreased uric acid and xanthines	Uric acid in serum, urine crystals, urine xanthine/ hypoxanthine	Uric acid in serum, urine. Purine and pyrimidines in urine by HPLC, gene studies nucleotides
Disorders of sterol and bile acid synthesis	Reduced cholesterol, deranged liver enzymes, cholestasis, deranged coagulation profile	Cholesterol in serum , lipid profile, liver function tests, coagulation factors, Vitamin levels (A, D, E, K)	Sterol assays in plasma: 7-dehydrocholesterol *etc* for bile acid synthetic defect – specific bile acids by FAB-MS, gene studies
Disorders of porphyrin and haem metabolism	Increased porphyrins in blood and urine	Porphyrins in blood and urine	Screening for Porphobilinogen in urine (Hoesch test, Watson-Schwartz test) qualitative/ quantitative Specific porphyrins in urine, stool or blood. Gene studies
Disorders of lipid and lipoprotein metabolism	Abnormal lipid profile, insulin	Lipid profile, insulin, liver enzymes	Enzyme assays, gene studies
Congenital disorders of glycosylation and other disorders of protein modification	Abnormal Liver function, insulin, coagulation factors	Liver function test and coagulation profile	Transferrin isoforms pattern by CZE, IEF or other method like HPLC. Enzyme assay (skin fibroblasts), gene studies
Lysosomal disorders	Anemia, pancytopenia, abnormal liver function, CK, urine GAG elevation, Cherry red spot / pigmentary changes in retina, dysostosis multiplex	Complete blood count, liver function, renal function, serum creatine kinase, Ophthalmic fundus exam, skeletal survey	Urine MPS and oligosaccharide screen, specific enzyme assay, plasma chitotriosidase, gene studies
Peroxisomal disorders	Deranged liver enzymes and VLCFA, abnormal lipids	Liver function tests, Renal functions, Lipid profile,	MRI brain, VLCFA analysis, Phytanic and pristanic acids, Plasmalogenes, gene studies Complementation studies,
Disorders of neuro-transmitter metabolism	Abnormal amino acid, organic acid profile, lipid profile, hyper prolactinemia, increased pipecolic acid	GELAK, S. Prolactin, S. uric acid, homocysteine, S. folic acid, vit B12 levels	Paired sampling – plasma and CSF amino acids, glucose and lactate, CSF neurotransmitters and pterins, gene studies

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Disease group/disorder	*Metabolic derangements**	*Basic metabolic tests for diagnosis*	*Diagnostic modalities of choice*
Disorders in the metabolism of vitamins and (non-protein) cofactors	Abnormal GELAAK, S. Prolactin,S. Uric Acid, hyperhomocysteinemia, with normal S. folic acid, vit B12 levels	Testing for GELAK, S. Prolactin, S. Uric Acid, homocysteine, S. folic acid, vit B12 levels	TMS, urine GC-MS, Biotinidase enzyme, gene studies
Disorders in the metabolism of trace elements and metals	Abnormal liver enzymes, Kayser Fletcher -ring, S.Ceruloplasmin, Serum and urinary copper (Wilson, Menke), S.Ferritin, S.uric acid (MoCD)	LFT, Eye exam for Kayser Fletcher-ring, testing for S.Ceruloplasmin, S.Ferritin, S.uric acid (MoCD)	Serum and urinary copper (Wilson, Menke), Hair shaft examination pili torti (Menke), gene studies
Disorders and variants in the metabolism of xenobiotics	Trimethylaminuria, White matter and other parenchymal abnormalities in brain (Sjogren Larson)	Specific punjent odor in urine (trimethylaminuria)	MRI brain,Skin fibroblast enzyme assay (SLS), free TMA in urine, gene studies

*SSIEM: Society for study of inborn errors of metabolism; mtDNA: mitochondrial DNA; nDNA: nuclear DNA; FAB-MS: Fast atom bombardment – mass spectrometry; MoCD: Molybdenum Cofactor Deficiency; TMA: trimethylaminuria; * Common only.*

require specialized tissues such as cultured skin fibroblasts, frozen liver biopsy or muscle biopsy specimen [6]. The diagnosis of these disorders relies heavily upon specific metabolite analysis using methods described below and molecular genetic testing involving sequencing of particular gene or group of genes.

Detection of Metabolites

These tests detect specific metabolites through simple chemical reactions with precision, sometimes alleviating the need for molecular genetic testing. Urine metabolic tests can be done easily in most laboratories. ***Table* II** depicts the preliminary simple bedside tests that can be used for the diagnosis of inborn metabolic errors. Among the specialized biochemical tests, three methods are described in more detail; whereas, other tests are mentioned in ***Table* III**.

Tandem mass spectrometry

Tandem mass spectrometry (TMS), also known as mass spectrometry-mass spectrometry (MS/MS) as two mass spectrometers are situated in tandem. This is a revolutionary technology devised in early 90s [17], which can detect multiple independent metabolites/analytes in a single test. The basic principle of TMS relies on the ionization and fragmentation of each molecule or metabolite into specific ions coupled with a robust detection system which is computerized to provide results [18]. ***Fig.* I** provides a simplified diagrammatic representation of MS/MS equipment [19]. A sample is first ionized and made to pass through a series of chambers, which serve specific purpose of further ionization, collision-induced dissociation and further ionization of daughter ions before reaching a detection chamber that recognizes individual ions based on specific mass-to-charge ratio. The computer then interprets the data and provides highly accurate results of each analyte. The utility of a TMS is multi-fold and its applications are expanding. It measures amino-acids, organic acids and fatty acids in form of their acyl-carnitine esters. This particular technology forms the core of expanded newborn screening throughout the developed world [20]. ***Table* IV** provides interpretations of few metabolites [21]. TMS does not screen for many IEMs such as mitochondriopathies, purine and pyrimidine disorders, neurotransmitters, congenital disorders of glycosylation (CDG) and very long chain fatty acids. TMS using dried blood spot is highly sensitive but not specific, and thus remains a screening test for majority of disorders. Its use for prenatal diagnosis is not recommended. Higher efficacy equipments such as liquid chromatography-MS/MS (LC-MS/MS) are now employed for better accuracy in biological samples, including CSF and plasma.

GC-MS urinalysis for organic acids

GC-MS is gas chromatography-mass spectrometry technology to detect metabolites specific for small molecule disorders (typically organic acids and related) in the urine [22]. The organic acids are volatile and tend to evaporate without preservation, which requires samples to be frozen. Detection of organic acids in urine should ideally be done in frozen sample or reliably if transported under ambient temperatures not exceeding 25°C. Transportation is stable when urine sample is put on a special grade filter paper that is able to soak in all metabolites and fix or stabilize it preventing it from evaporation [22]. They can be eluted back from filter paper into liquid solution using simple techniques.

TABLE III BASIC METABOLIC TESTS IN URINE AND THEIR INTERPRETATION

Test	*Disorder screened*	*Analyte, metabolite or color*
Multistix reagent test	Multiple disorders	Blood, leukocytes, nitrites, glucose, ketones (Acetoacetic acid), pH, specific gravity,creatinine, bilirubin and urobilinogen
Reducing substances (non-glucose)	Fructose intolerance (HFI, Fructosemia)	Fructose
	Galactosemia (GALT, GALK or epimerase deficiency)	Galactose
	Pentosuria	Xylose
	Alkaptonuria	Homogentisic acid
	Tyrosinemia 1, 2	4-Hydroxyphenyl pyruvic acid
Dinitrophenyl-hydrazine (DNPH) test	Maple Syrup Urine Disease MSUD	Branched chain 2-ketoacids (2-keto isovaleric, 2-keto isocaproic, 2-keto 3 methylvaleric acids)
	Phenylketonuria	Phenylpyruvic acid
	Liver disease, tyrosinemia 1 / 2, tyrosiluria, Histidinemia, methionine malabsorption (Oasthouse syndrome)	4-Hydroxyphenyl pyruvic acid
	Ketosis	Acetone
Ferric chloride test	Phenylketonuria	Phenylketones - phenylpyruvic acid (greenish-blue)
	Histidinemia, Pheochromocytoma, Alkaptonuria	Blue green
	MSUD, Foriminotransferase deficiancy	Grey green
	Salicylates, Methionine malabsorption, DKA	Purple
	Phenothiazines, Para amino salicylic acid	Blue purple
Nitrosonaphthol test	PKU, Direct hyperbilirubinemia, Tyrosinemia, INH, L-Dopa	Green
Cyanide nitroprusside test	Liver disease, tyrosinemia 1 / 2/transient, tyrosiluria, Histidinemia, methionine mala-bsorption (Oasthouse syndrome), TPN	Tyrosine and 4-hydroxylated phenolic acids (tyrosine analogs like 4-hydroxy-phenyl pyruvic acid, 4-hydroxy-phenyllactate, and 4-hydroxy-phenylacetate)
	Cystinuria	Cystine
	Homocystinuria, severe dietary or inherited cobalamin/folate deficiency,	Homocysteine
Ehrlich's Aldehyde test	Porphyria	Porphobilinogen, urobilinogen
Sulfite test	Sulfite oxidase, Molybdenum cofactor deficiency	Sulfites in urine
Alcian blue, Toluidine blue test - MPS screen test on filter paper	Mucopolysaccharide	Blue color on filter paper urine spot

Interpretation of results of urine organic acid analysis by GC-MS requires expertise and biochemical background training, and thus should be taken up only by specialized genetic laboratories or institutes. The modality can also measure metabolites in bile, plasma and other body fluids including postmortem samples when there is a failure to collect urine.

Ultra/high performance liquid chromatography (U/HPLC) or ion-exchange chromatography

The measurement of specific amino acids in body fluids such as plasma/serum, urine or CSF is being utilized widely for diagnosing many amino-acid disorders such as phenylketonuria, tyrosinemia type 1/2/3, maple syrup urine disease, and homocystinuria (methionine as well as homocysteine which requires a different algorithm for identification) [23]. There are multiple methods for quantifying amino acids – chromatographic as well as electrophoresis. Most laboratories currently employ either HPLC or UPLC technique because of the relative ease and high specificity. All samples (plasma not whole blood, urine or CSF) for chromatography or

TABLE IV INTERPRETATION OF TANDEM MASS SPECTROMETRY FOR INBORN ERRORS OF METABOLISM

Category	*Analyte*	*Derangement*	*Interpretation*
Amino acids	Citrulline	Elevated	Citrullinemia type1 or 2, Arginino-succinic aciduria
	Methionine	Elevated	Homocystinuria
	Leucine+Isoleucine, Valine	Elevated	Maple Syrup Urine Disease
	Phenylalanine	Elevated	Phenylketonuria, Non-PKU pterin disorders
	Tyrosine	Elevated	Hereditary Tyrosinemia type 1/2/3
	Glycine	Elevated	Ketotic/Non-ketotic hyperglycinemia
	Alanine	Elevated	Suggestive of mitochondriopathy but not diagnostic
	Glutamine	Elevated	Hyperammonemia
Acyl-carnitines			
Carnitine	Free carnitine (C0)	Reduced	Primary/Secondary carnitine deficiency
	Free carnitine (C0)	Elevated	Carnitine Palmitoyl Transferase 1 deficiency, iatrogenic
Organic acids	Propionyl carnitine (C3)	Elevated	Methylmalonic acidemia, Propionic acidemia, Vitamin B12 deficiency, drugs
	Butyryl carnitine (C4)	Elevated	Ethylmalonic aciduria, Ethylmalonic encephalopathy, Short chain acyl CoA dehydrogenase deficiency, Isobutyryl-CoA dehydrogenase deficiency
	Isovaleryl carnitine (C5)	Elevated	Isovaleric acidemia
	Tiglyl carnitine (C5:1)	Elevated	Beta-ketothiolase deficiency
	3-hydroxyisovaleryl-carnitine OR 2-methyl-3-hydroxybutyryl carnitine (C5OH)	Elevated	Multiple carboxylase deficiency (Holocarboxylase deficiency, Biotinidase deficiency), HMG CoA lyase deficiency, 3-Methyl crotonyl CoA carboxylase deficiency, Beta-ketothiolase deficiency, 2-Methyl 3 hydroxy butyric aciduria, 3-Methyl glutaconic aciduria
	Glutaryl carnitine (C5DC)	Elevated	Glutaric aciduria type 1 or 2 (multiple acyl co-A dehydrogenase deficiency)
Fatty acids: Medium chain acyl carnitines	Hexanyl carnitine (C6) Octanoyl Carnitine (C8) Decenoyl carnitine (C10:1) Decanoyl carnitine (C10)	Elevated	Medium-chain acyl-CoA dehydrogenase deficiency
Fatty acids: Very long chain acyl carnitines	Tetradecanoyl (C14), Tetradecenoyl-(C14:1) and Tetradecadienoyl-carnitine (C14:2)	Elevated	Very long chain acyl CoA dehydrogenase deficiency
Fatty acids: Long chain acyl carnitines	Hexadecanoyl- (C16) Octadecanoyl- (C18) Octadecenoyl –(C18:1) and Octadecadienoyl- carnitine (C18:2)	Elevated	Carnitine palmitoyl transferase II deficiency
Fatty acids: Long chain hydroxyl acyl carnitines	3-hydroxyhexadecenoyl-(C16:1-OH),3-hydroxyhexadecanoyl-(C16-OH), 3-hydroxyoctadecenoyl-(C18:1-OH) and 3-hydroxy octadecanoyl-carnitine (C18-OH)	Elevated	Long-chain 3-OH acyl-CoA dehydrogenase deficiency, Trifunctional protein deficiency
Fatty acids: Multiple	C4–C18 saturated and unsaturated species	Elevated	Multiple acyl CoA dehydrogenase deficiency (Glutaric aciduria type 2)

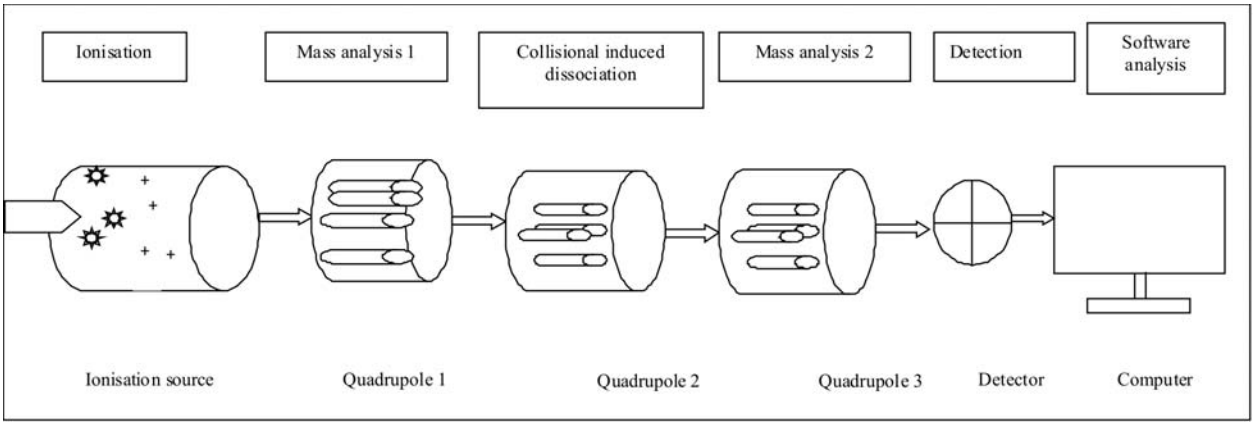

FIG. 1 *Basic steps of tandem mass spectrometry*.

electrophoresis should be transported in frozen conditions using dry ice. Samples exposed to high temperatures and harsh conditions would destroy the amino acids and give erroneous or inaccurate results. Samples can also be collected in a sulfosalicylic acid (SSA) pretreated vial to ensure stability. CSF samples should be paired with plasma samples for accurate interpretation. Diagnosis of non-ketotic hyperglycinemia will be made when both CSF and plasma levels of glycine are elevated. Isolated elevation of glycine may be due to other non-genetic causes such as perinatal asphyxia or bloody tap (due to contamination of CSF with blood). Interpretation for some common and important metabolite/amino acid derangement is provided in ***Table* III**. Detection of amino acids qualitatively in the urine (and sometimes both urine as well as plasma) has also been used traditionally for the diagnosis amino acid disorders. The test is done by a thin-layer chromatography technique that is a simple test using filter paper. The pattern of excretion of amino acids is interpreted to get to a diagnosis.

Other Metabolic Investigations

Neurotransmitter assays: This is a specialized test performed only on CSF using sophisticated LC-MS/MS and GCMS technique [10] that only few laboratories have standardized and thus authorized to perform.

Serum transferrin isoforms for glycosylation pattern: This is a widely used screening test for CDGs, the diagnosis of which requires genetic testing. The test was initially established using iso-electric focusing (IEF) [24] followed by capillary zone electrophoresis (CZE) in the nineties [25]. There are two abnormal patterns of transferrins identified in CDGs leading to their classification into either CDG type 1 (showing a type 1 pattern of derangement of transferrin isoforms) or type 2. However, not all CDGs would show up with these patterns [26]. The test can also be done using HPLC, MALDI-TOF-MS/MS or LC-MS/MS [27].

Metabolic biomarkers: Testing for chitotriosidase, CCL18/PARC, heparin thrombin cofactor ii, lyso GB3 are available in few specialized laboratories. Multiple biomarkers are in use for diagnosis and prognosis of many metabolic disorders. A holistic approach is recommended to detect and interpret certain (specific) pattern of metabolites in body fluids to make a metabolic diagnosis. This new system also known as the metabolomics approach is rapidly advancing and holds promise for the future [28]

Molecular Genetic Testing

The molecular genetic test entails testing of either single or multiple genes together depending upon the suspicion of the disease. For disorders where a specific diagnosis is already made using other techniques such as GALT deficiency, only one gene (*GALT*) may be tested. However, for other disorders like mitochondrial disorders, multiple genes are to be tested together for lack of evidence towards any specific genes (barring few exceptions). There are two methods employed for molecular genetic testing: a traditional, well established and more precise method of Sanger sequencing [29], and a more advanced method known as Next generation sequencing (NGS). Both methods allow for sequencing of genes, determining the sequence of base pairs in the DNA of the exons/exon-intron boundaries or coding regions of a gene. However, Sanger sequencing is time consuming, laborious and expensive as it is performed fragment wise, one by one for each fragment of a gene. The NGS utilizes the multiplexing of all the sequencing fragments and thus has the capacity to sequence any number of DNA fragments simultaneously. The NGS can

KEY MESSAGES

- Inborn errors of metabolism (IEM) may present at any age (antenatal, birth, infancy, childhood or adulthood), with common illnesses such as vomiting, respiratory distress, lethargy or even seizures.
- A recurrence of similar illness in the past or in family should raise suspicion for an IEM. Presence of consanguinity must be asked in all cases.
- In an acutely sick child, IEMs should be suspected at the same time as other diagnoses like infection, trauma, poisoning. Prompt testing with basic metabolic tests would save time and benefit the child with early treatment possibilities.
- Communication with metabolic (biochemical and genetic) laboratory and metabolic specialist is beneficial for appropriating the testing methodology as well as in acute management.

sequence from one gene to whole exome or even genome which is about 3 billion base pairs. By multiplexing the sequencing there is considerable saving of time as well as cost. It is now becoming the standard diagnostic methodology in most of genetic laboratories, including for metabolic disorders.

Pitfalls

An entire battery of test has been developed over the years to diagnose IEMs. The basic and advanced metabolic test would diagnose majority of IEMs, except few. TMS can detect only three groups of disorders (fatty acid oxidation, organic and few amino acidurias) and not mitochondrial disorders. A biochemical test may show normal metabolite levels as they are not always deranged. In such cases, the metabolic tests should be repeated during acute sickness.

CONCLUSION

Inborn errors of metabolism are disorders often encountered in pediatric emergencies presenting with common symptoms. As many of them are treatable, a high index of suspicion can lead to their early recognition. Early and accurate treatment is the key to a fruitful outcome. IEMs can be screened by basic biochemical tests. Appropriate samples should also be collected and stored at the time of acute illness for advanced genetic tests, which can be carried out later. All IEMs have a genetic basis: hence, an accurate genetic counseling should be provided to the families for prevention in future births. With knowledge of the basic and advanced metabolic tests, the pediatrician would be empowered to diagnose and treat most IEMs.

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