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SHORT REVIEW

Screening for Inborn Errors of Metabolism

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Abstract

Inborn errors of metabolism (IEM) are a heterogeneous group of monogenic diseases that affect the metabolic pathways. The detection of IEM relies on a high index of clinical suspicion and co-ordinated access to specialized laboratory services. Biochemical analysis forms the basis of the final confirmed diagnosis in several of these disorders.

1. The investigations fall into four main categories
2. General metabolic screening tests
3. Specific metabolite assays
4. Enzyme studies
5. DNA analysis

The first approach to the diagnosis is by a multi-component analysis of body fluids in clinically selected patients, referred to as metabolic screening tests. These include simple chemical tests in the urine, blood glucose, acid-base profile, lactate, ammonia and liver function tests. The results of these tests can help to suggest known groups of metabolic disorders so that specific metabolites such as amino acids, organic acids, etc. can be estimated. However, not all IEM needs the approach of general screening. Lysosomal, peroxisomal, thyroid and adrenal disorders are suspected mainly on clinical grounds and pertinent diagnostic tests can be performed. The final diagnosis relies on the demonstration of the specific enzyme defect, which can be further confirmed by DNA studies.

Keywords: *Inborn errors of metabolism (IEM); metabolic screening; DNA analysis.*

"The goal of newborn screening is early detection of children at increased risk for selected metabolic or genetic diseases so that medical treatment can be promptly initiated to avert metabolic crises and prevent irreversible neurological and developmental sequelae."

Newborn Screening in New York

A Guide for Health Professionals 1991.

Inborn errors of metabolism (IEM) necessitates the attention of pediatricians and neonatologists as a cause of illness during the neonatal period, as many disorders are treatable if detected early, which could lead to the early initiation of therapy. Despite some of them being untreatable, it is vital to establish the diagnosis in the index case, in order to facilitate prenatal diagnosis in subsequent pregnancies [1].

Some of the basic criteria for determining which inherited disorders for newborn screening include [2]:

- The disorder has a relatively ***high incidence*** so that the cost per diagnosed individual is reasonable;
- An effective and ***not overly expensive*** treatment is available;
- A relatively ***inexpensive screening test*** is available that is suitable for ***high volume testing*** (preferably automatable);
- The screening test has a very ***high sensitivity*** (i.e. a very low rate of false negatives) and ***high specificity*** (i.e. low rate of false positives which require expensive follow-up).

Criteria for Newborn Screening

- Disorder produces irreversible damage before onset of symptom;

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- Treatment is effective if begun early;
- Natural history of disorder is known

Biochemical diagnosis involves the following screening tests:

1. General metabolic screening tests
2. Specific metabolite assays
3. Enzyme studies
4. DNA analysis

The first approach to the diagnosis is by a multi-component analysis of body fluids in clinically selected patients, referred to as metabolic screening tests. These include simple chemical tests in the urine, blood glucose, acid-base profile, lactate, ammonia and liver function tests. The results of these tests can help to suggest known groups of metabolic disorders so that specific metabolites such as amino acids, organic acids, etc. can be estimated However, not all IEM needs the approach of general screening. Lysosomal, peroxisomal, thyroid and adrenal disorders are suspected mainly on clinical grounds and pertinent diagnostic tests can be performed. The final diagnosis relies on the demonstration of the specific enzyme defect which can be further confirmed by DNA studies [3].

Urine screening tests

Several screening tests are available to detect the abnormal metabolites excreted in the urine in many IEM. The initial observation of the urine sample can help to identify unusual color or odors, if present (Table 1). The ferric chloride test is generally used to screening for phenylketonuria (PKU). However, several compounds react with ferric chloride to give various colors. Moreover, this test may not be positive in neonates with PKU as they excrete very little phenylpyruvate.

The dinitrophenylhydrazine (DNPH) test detects α-ketoacids and is useful for screening PKU and maple syrup urine disease. A positive test should be followed up with amino acid and organic acid analyses of the blood and urine. Commercially available reagent strips can be used to detect ketone bodies in the urine. These strips are most sensitive to acetoacetic acid but can also react with acetone and butanone.

The cyanide nitroprusside test detects any compound containing the sulfhydryl group and is used to screen for homocystinuria and cystinuria. Several drugs like synthetic penicillins, D-pencilamine, and N-acetylcysteine give a positive reaction. Reducing substances in the urine can be detected by Benedict's test. A positive Benedict's test should be followed by testing for glucose using a strip (Uristix), and when associated with a negative strip test for glucose it indicates that the reducing substance is not glucose. These reducing substances can be further differentiated by thin-layer chromatography. False positive tests are produced by large amounts of uric acid, ascorbic acid, cephalosporins, ampicillin, and other drugs [4].

Therefore, the diagnosis and early detection of IEM form an import segment of the clinical chemistry laboratory. Children with symptoms including metabolic acidosis, jaundice, hepatosplenomegaly, recurrent vomiting, hypoglycemia, hyperammonemia, mental retardation of unknown causes, language retardation, seizures and unconsciousness need screening for IEM. The availability of techniques like tandem mass spectrometry (MS/MS) in newborn screening makes it possible to screen for a wide range of previously unscreened IEM, using a single test.

Therefore, screening of IEM forms an important part of a clinical chemistry laboratory. Some of the useful tests for IEM using urine samples, like plasma aminoacid abnormalities in disorders of aminoacid metabolism, typical urine organic acid profiles in some organic acidurias and the use of enzymes in the diagnosis of lysosomal disorders are listed in the Tables 1-4.

Table 1.
Qualitative urine tests for Inborn Errors of Metabolism

Test	Result	Compound detected	Disorder
Odor	Musty	Phenylacetate	Phenylketonuria
	Maple syrup	2-Oxoisocaproate, 2-oxo-3-methyl valerate	Maple syrup urine Disease (MSUD)
	Sweaty feet	Isovalerate	Isovaleric acidemia
	Cat urine	3-Hydroxyisovalerate	3-Methylcrotonyl glycinuria
Ferric chloride test	Blue-green color	Phenylpyruvate Imidazole pyruvate Xanthurenic acid	Phenylketonuria Histidinemia Xanthurenic aciduria
	Transient blue-green	Homogentisate	Alkaptonuria
	Green-gray	Branched-chain oxoacids	MSUD
	Green color	p-hydroxyphenyl pyruvate	Tyrosinemia, types 1&2
Dinitro-phenyl hydrazine (DNPH) test	Golden yellow precipitate	Phenylpyruvate 2-Oxoisovalerate 2-oxoisocaproate 2-oxo-3-methyl valerate	Phenylketonuria MSUD
		4-Hydroxyphenyl pyruvate	Tyrosinemia, types 1&2
		Pyruvate	Lactic acidosis
Cyanide Nitro-prusside test	Magenta color	Cystine	Cystinuria, hyperarginemia,
		Homocystine	Homocystinuria
Benedict's test	Brick red precipitate	Glucose Galactose Fructose	Diabetes, Fanconi synd Galactosemia Fructose intolerance, Essential fructosuria
		Xylose	Pentosuria
		4-Hydroxypheny pyruvate	Tyrosinemias, types 1&2
		Oxalic acid	Hyperoxaluria
	Brown ppt	Homogentisate	Alkaptonuria
Strip test for ketones (Ketostix)	Positive	Acetone, butanone, acetoacetate, 2-methylacetoacetate	3-Oxothiolase defect,popionic acidemia, methylmalonic acidemia
Berry spot test	Purple ring	Mucopolysaccharides	Mucopolysaccharidoses

Table 2.

Inborn errors of amino acid metabolism

Plasma amino acid increased	Disease	Defective enzyme/ cofactor
Aspartyl glucosamine	Aspartylglucosaminuria	Aspartylglycosaminidase
Alanine	Elevated in pyruvate/ lactate disorders, hyperammonemia	-
Arginine	Hyperargininemia	Arginase
Argininosuccinic Acid	Argininosuccinic aciduria	Argininosuccinate lyase
Citrulline	Citrullinemia Argininosuccinic aciduria Pyruvate carboxylase defect	Argininosuccinate synthetase Argininosuccinate lyase Pyruvate carboxylase
Cystathionine	Cystathioninemia	Cystathionine g-lyase
Glycine	Nonketotic hyperglycinemia Certain organic acidemias	Glycine cleavage system -
Histidine	Histidinemia	Histidase
Homocysteine	Homocystinuria	Cystathionine b-synthase, Methylene tetrahydrofolate reductase
Isoleucine, Leucine, Valine	Maple syrup urine disease	Branched-chain ketoacid dehydrogenase
Lysine	Persistent hyperlysinemia	2-amino adipic semialdehyde synthase
Methionine	Hypermethioninemia Homocystinuria Tyrosinemia I	Methionine adenosyl transferase Cystathionine b-synthase Fumarylacetoacetate hydrolase
Ornithine	Hyperornithinemia with gyrate atrophy HHH syndrome	Ornithine d-aminotransferase Ornithine transporter
Phenylalanine	Phenylketonuria	Phenylalanine hydroxylase/ Tetrahydrobiopterin defect
Proline	Hyperprolinemia type I Hyperprolinemia type II	Proline oxidase D^1-pyrroline 5-carboxylate dehydrogenase
Tyrosine	Tyrosinemia, type I Tyrosinemia, type II Tyrosinemia type III	Fumarylacetoacetate hydrolase Tyrosine aminotransferase 4-hydroxyphenylpyruvate dioxygenase

Table 3.

Organic acid profiles in urine at some organic acidurias

Elevated urine organic acids	Disease	Enzyme defect
2-Ketoisocaproate, 2-hydroxycaproate, 2-keto-3-methylvalerate, 2-ketoisovalerate, 2-hydroxyisovalerate	Maple syrup urine disease	Branched-chain a-ketoacid dehydrogenase
3-Hydroxy 3-methylglutarate, 3-methylglutaconate, 3-hydroxyisovalerate	HMG CoA lyase deficiency	3-hydroxy 3-methyl glutaryl (HMG) CoA lyase
Isovalerylglycine, 3-hydroxyisovalerate, lactate, 3-hydroxybutyrate, acetoacetate	Isovaleric aciduria	Isovaleryl-CoA Dehydrogenase
3-Methylcrotonate, 3-methylcrotonylglycine, 3-hydroxyisovalerate	3-Methyl crotonyl glycinuria	3-Methylcrotonyl CoA carboxylase
3-Methylcrotonate, 3-methylcrotonylglycine, 3-hydroxyisovalerate, propionate, 3-hydroxypropionate, methylcitrate, tigylglycine, lactate, 3-hydroxy butyrate, acetoacetate	Multiple carboxylase deficiency	Holocarboxylase synthetase Biotinidase
Methylmalonate, methylcitrate, 3-hydroxybutyrate acetoacetate	Methylmalonic aciduria	Methylmalonyl-CoA mutase or cobalamin defects
Propionate, 3-hydroxypropionate, propionylglycine, methylcitrate, tigylglycine, 3-hydroxybutyrate, acetoacetate	Propionic aciduria	Propionyl CoA Carboxylase
Glutarate, 3-hydroxyglutarate	Glutaric aciduria, type I	Glutaryl-CoA Dehydrogenase
Glutarate, 2-hydroxyglutarate, ethylmalonate, adipate, suberate, sebacate, dodecanedioate, isovalerylglycine, hexanoylglycine	Glutaric aciduria, type II	Electron transfer flavoprotein dehydrogenase
N-Acetylaspartate	Canavan disease	Aspartoacylase

Table 4.

Enzymes in the diagnosis of lysosomal storage disorders

Disorder	Affected Enzyme	Tissue
Sphingolipidoses		
GM_1 gangliosidosis	b-Galactosidase	S, L, F
GM_2 gangliosidoses-Tay Sach Sandhoff	b-Hexosaminidase A	S, L, F
Metachromatic leukodystrophy	Arylsulfatase A	S, L, F
Krabbe leukodystrophy	Galactocerebrosidase	L, F
Fabry disease	a-Galactosidase A	S, L, F
Gaucher disease	Glucocerebrosidase	L, F
Niemann-Pick disease, A &B	Sphingomyelinase	L, F
Farber lipogranulomatosis	Ceramidase	L, F
Mucopolysaccharidoses		
Hurler disease (MPS I-H)	a-L-iduronidase	S, L, F
Scheie disease (MPS I-S)	a-L-iduronidase	S,L, F
Hunter disease (MPS II)	Iduronate 2-sulfatase	L, F
Sanfilippo, A (MPS IIIA)	Heparan N-sulfatase	L, F
Sanfilippo, B (MPS IIIB)	a-N-Acetylglucosaminidase	L, F
Sanfilippo, C (MPS IIIC)	Acetyl CoA: a-glucosaminide acetyltransferase	L, F
Sanfilippo , D (MPS IIID)	N-Acetylglucosamine-6-sulfatase	L, F
Morquio, A (MPS IVA)	N-Acetyl galactosamine-6-sulfatase	L, F
Morquio , B (MPS IVB)		L, F
Maroteaux-Lamy (MPS VI)	b-Galactosidase	L, F
Sly syndrome (MPS VII)	N-Acetylgalactosamine 4-sulfatase b-Glucuronidase	S, L, F
Glycoproteinoses		
a-Fucosidosis	a-Fucosidase	S, L, F
a-Mannosidosis	a-Mannosidase	L, F
b-Mannosidosis	b-Mannosidase	L, F
Sialidosis, type I	a-Neuraminidase	L, F
Galactosialidosis	a-Neuraminidase & b-Galactosidase	L, F
Schindler disease	a-N-acetylgalactosaminidase	L, F
Aspartylglucosaminuria	Aspartylglucosaminidase	L, F
I-cell disease & Pseudo-Hurler Polydystrophy (Mucolipidosis, II & III)	All lysosomal enzymes elevated in plasma except b-glucosidase	S, F
Other storage disorders		
Wolman disease	Acid esterase	L,F
Pompe disease	Acid maltase	Muscle

Abbreviations: ***S:*** *Serum;* ***L:*** *Leukocytes;* ***F:*** *Fibroblasts*

Molecular genetic studies

Molecular genetic studies are increasingly relied upon to confirm the diagnosis of many IEM. With a few exceptions, in all inherited metabolic diseases for which the gene responsible has been isolated and disease-producing mutations characterized, no single mutation has been identified which accounts for all the cases. The patient may have mutant alleles that have not yet been characterized or they may be so rare that testing for them routinely may not be economically feasible. Hence, although the detection of a certain mutation in the tissue from a patient is considered a strong support for diagnosis, failure to demonstrate the presence of the mutation does not rule out the diagnosis [5].

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