

Approach to Inborn Errors of Metabolism Presenting in the Neonate

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Abstract

Inborn errors of metabolism (IEM) are an important cause of acute illness in newborns. Presentation may mimic common neonatal conditions such as sepsis. Prompt detection requires a high index of suspicion and the early measurement of biochemical markers such as blood ammonia. Diagnosis is important not only for treatment but also for genetic counselling. Guidelines for diagnosis and early management of IEM presenting in the neonatal period are described.

Key words: Inborn errors of metabolism, encephalopathy, hyperammonemia

AIIMS Protocols

Inborn errors of metabolism (IEM) are disorders in which there is a block at some point in the normal metabolic pathway caused by a genetic defect of a specific enzyme. The number of diseases in humans known to be attributable to inherited point defects in metabolism now exceeds 500.¹ While the diseases individually are rare, they collectively account for a significant proportion of neonatal and childhood morbidity and mortality. Diagnosis is important not only for treatment and prognostication but also for genetic counselling and antenatal diagnosis in subsequent pregnancies.

Clinical Presentation:

Severe illness in the newborn, regardless of the underlying cause, tends to manifest with non-specific findings, such as poor feeding, drowsiness, lethargy, hypotonia and failure to thrive. IEM should be considered in the differential diagnosis of any sick neonate along with common acquired causes such as sepsis, hypoxic-ischemic encephalopathy, duct-dependant cardiac lesions, congenital adrenal hyperplasia and congenital infections. Clinical pointers towards an underlying IEM include²:

- Deterioration after a period of apparent normalcy
- Parental consanguinity
- Family history of unexplained neonatal deaths
- Rapidly progressive encephalopathy and seizures of unexplained cause
- Severe metabolic acidosis
- Persistent vomiting
- Peculiar odor (urine, cerumen)
- Acute fatty liver or HELLP (hemolysis, elevated liver enzymes & low platelet counts) during pregnancy: seen in women carrying fetuses with long-chain-3-hydroxyacyl-coenzyme dehydrogenase deficiency (LCHADD).

Table 1 describes examination findings that may provide a clue to the underlying IEM. Patterns of presentation include^{2,3}:

- 1) *Encephalopathy with or without metabolic acidosis*: encephalopathy, seizures, and tone abnormalities are predominant presenting features of organic acidemias, urea cycle defects and congenital lactic acidosis. Isolated intractable seizures are prominent in pyridoxine dependency, pyridoxal phosphate dependency, folinic acid responsive seizures, and glucose transporter 1 deficiency.⁴
- 2) *Acute liver disease*: This could manifest as-
 - Jaundice alone- Gilbert syndrome, Crigler-Najjar syndrome
 - Hepatic failure (jaundice, ascites, hypoglycemia, coagulopathy)- Tyrosinemia, galactosemia, neonatal hemochromatosis, glycogen storage disease type IV.
 - Neonatal cholestasis: alpha-1 antitrypsin deficiency, Niemann-Pick disease type C.
 - Hypoglycemia: persistent and severe hypoglycemia may be an indicator of an underlying IEM. Hypoglycemia is a feature of galactosemia, fatty acid

oxidation defects, organic acidemias, glycogen storage disorders and disorders of gluconeogenesis.

- 3) *Dysmorphic features*: seen in peroxisomal disorders, pyruvate dehydrogenase deficiency, congenital disorders of glycosylation (CDG), and lysosomal storage diseases. Some IEMs may present with non-immune hydrops fetalis; these include lysosomal storage disorders and CDG.
- 4) *Cardiac disease*: cardiomyopathy is a prominent feature in some IEM including fatty acid oxidation defects, glycogen storage disease type II and mitochondrial electron transport chain defects.

Investigations

Metabolic investigations should be initiated as soon as the possibility is considered. The outcome of treatment of many IEM especially those associated with hyperammonemia is directly related to the rapidity with which problems are detected and appropriate management instituted.

First line investigations (metabolic screen):

The following tests should be obtained in ALL babies with suspected IEM.

- 1) Complete blood count: (neutropenia and thrombocytopenia seen in propionic and methylmalonic academia)
- 2) Arterial blood gases and electrolytes
- 3) Blood glucose
- 4) Plasma ammonia (Normal values in newborn: 90-150 $\mu\text{g/dl}$ or 64-107 $\mu\text{mol/L}$)
- 5) Arterial blood lactate (Normal values: 0.5-1.6 mmol/L)
- 6) Liver function tests
- 7) Urine ketones
- 8) Urine reducing substances.
- 9) Serum uric acid (low in molybdenum cofactor deficiency).

Figure 1 gives the algorithmic approach to a newborn with suspected IEM. Disease category can be diagnosed based on blood ammonia, blood gas analysis and urine ketone testing. Hyperammonemia without acidosis is caused by urea cycle defects. Metabolic acidosis with or without hyperammonemia is a feature of organic acidemias and fatty acid oxidation defects. Figure 2 explains the algorithmic approach to neonate with persistent hypoglycemia and suspected underlying IEM. Table 2 explains the categorization of IEM based on simple metabolic screening tests.

Second line investigations (ancillary and confirmatory tests)

These tests need to be performed in a targeted manner, based on presumptive diagnosis reached after first line investigations:

- 1) Gas chromatography mass spectrometry (GCMS) of urine- for diagnosis of organic acidemias.
- 2) Plasma amino acids and acyl carnitine profile: by tandem mass spectrometry (TMS)- for diagnosis of organic acidemias, urea cycle defects, aminoacidopathies and fatty acid oxidation defects.
- 3) High performance liquid chromatography (HPLC): for quantitative analysis of amino acids in blood and urine; required for diagnosis of organic acidemias and aminoacidopathies.
- 4) Lactate/pyruvate ratio- in cases with elevated lactate.
- 5) Urinary orotic acid- in cases with hyperammonemia for classification of urea cycle defect.
- 6) Enzyme assay: This is required for definitive diagnosis, but not available for most IEM's. Available enzyme assays include: biotinidase assay- in cases with suspected biotinidase deficiency (intractable seizures, seborrheic rash, alopecia); and . GALT (galactose 1-phosphate uridyl transferase) assay- in cases with suspected galactosemia (hypoglycemia, cataracts, reducing sugars in urine).
- 7) Neuroimaging: MRI may provide helpful pointers towards etiology while results of definitive investigations are pending. Some IEM may be associated with structural malformations e.g. Zellweger syndrome has diffuse cortical migration and sulcation abnormalities. Agenesis of corpus callosum has been reported in Menke's disease, pyruvate decarboxylase deficiency and nonketotic hyperglycinemia.⁵ Examples of other neuroimaging findings in IEM include:
 - Maple syrup urine disease (MSUD): brainstem and cerebellar edema
 - Propionic & methylmalonic acidemia: basal ganglia signal change
 - Glutaric aciduria: frontotemporal atrophy, subdural hematomas
- 8) Magnetic resonance spectroscopy (MRS): may be helpful in selected disorders E.g. lactate peak elevated in mitochondrial disorders, leucine peak elevated in MSUD.
- 9) Urine for alpha-amino adipic semialdehyde, - elevated in pyridoxine dependent seizures. (test not available in India yet).⁴
- 10) Electroencephalography (EEG): some EEG abnormalities may be suggestive of particular IEM; e.g. comb-like rhythm in MSUD, burst suppression in NKH and holocarboxylase synthetase deficiency.⁶
- 11) Plasma very long chain fatty acid (VLCFA) levels: elevated in peroxisomal disorders.

12) Mutation analysis when available.

13) CSF aminoacid analysis: CSF Glycine levels elevated in NKH.

Precautions to be observed while collecting samples

1. Should be collected before specific treatment is started or feeds are stopped, as may be falsely normal if the child is off feeds.
2. Samples for blood ammonia and lactate should be transported in ice and immediately tested. Lactate sample should be arterial and should be collected after 2 hrs fasting in a preheparinized syringe. Ammonia sample is to be collected approximately after 2 hours of fasting in EDTA vacutainer. Avoid air mixing. Sample should be free flowing.
3. Detailed history including drug details should be provided to the lab. (sodium valproate therapy may increase ammonia levels).

Samples to be obtained in infant with suspected IEM when diagnosis is uncertain and death seems inevitable (Metabolic autopsy)^{7,8}

1. Blood: 5-10 ml; frozen at -20°C ; both heparinized (for chromosomal studies) and EDTA (for DNA studies) samples to be taken
2. Urine: frozen at -20°C
3. CSF: store at -20°C
4. Skin biopsy: 3 X 2 mm full thickness in culture medium or saline with glucose. Store at $4-8^{\circ}\text{C}$. Do not freeze.
5. Liver and muscle biopsy: for histopathology, electron microscopy, and enzyme studie.
6. Kidney and heart biopsy: as indicated.
7. Clinical photograph (in cases with dysmorphism)
8. Infantogram (in cases with skeletal abnormalities)

Treatment

In most cases, treatment needs to be instituted empirically without a specific diagnosis. The metabolic screen helps to broadly categorize the patient's IEM (e.g. urea cycle defect, organic academia, congenital lactic acidosis etc), on the basis of which, empirical treatment can be instituted.

Aims of treatment

1. To reduce the formation of toxic metabolites by decreasing substrate availability (by stopping feeds and preventing endogenous catabolism)
2. To provide adequate calories

3. To enhance the excretion of toxic metabolites.
4. To institute co-factor therapy for specific disease and also empirically if diagnosis not established.
5. Supportive care- treatment of seizures (avoid sodium valproate – may increase ammonia levels), maintain euglycemia and normothermia, fluid, electrolyte & acid-base balance, treatment of infection, mechanical ventilation if required.

Management of hyperammonemia^{9,10}

- 1) Discontinue all feeds. Provide adequate calories by intravenous glucose and lipids. Maintain glucose infusion rate 8-10 mg/kg/min. Start intravenous lipid 0.5 g/kg/day (up to 3g/kg/day). After stabilization gradually add protein 0.25 g/kg till 1.5 g/kg/day.
- 2) Dialysis is the only means for rapid removal of ammonia, and hemodialysis is more effective and faster than peritoneal dialysis, however peritoneal dialysis may be more widely available and feasible. Exchange transfusion is not useful.
- 3) Alternative pathways for nitrogen excretion:-
Sodium benzoate (IV or oral)- loading dose 250 mg/kg then 250-400 mg/kg/day in 4 divided doses. (Intravenous preparation not available in India).
Sodium phenylbutyrate (not available in India)-loading dose 250 mg/kg followed by 250-500 mg/kg/day.
L-arginine (oral or IV)- 300 mg/kg/day (Intravenous preparation not available in India)
L-carnitine (oral or IV)- 200 mg/kg/day
- 4) Supportive care: treatment of sepsis, seizures, ventilation. Avoid sodium valproate.

Acute management of newborn with suspected organic acidemia¹¹

- 1) The patient is kept nil per orally and intravenous glucose is provided.
- 2) Supportive care: hydration, treatment of sepsis, seizures, ventilation.
- 3) Carnitine: 100 mg/kg/day IV or oral.
- 4) Treat acidosis: Sodium bicarbonate 0.35-0.5mEq/kg/hr (max 1-2mEq/kg/hr)
- 5) Start Biotin 10 mg/day orally.
- 6) Start Vitamin B12 1-2 mg/day I/M (useful in B12 responsive forms of methylmalonic acidemias)
- 7) Start Thiamine 300 mg/day (useful in thiamine-responsive variants of MSUD).
- 8) If hyperammonemia is present, treat as explained above.

Management of congenital lactic acidosis

- 1) Supportive care: hydration, treatment of sepsis, seizures, ventilation. Avoid sodium valproate.
- 2) Treat acidosis: sodium bicarbonate 0.35-0.5mEq/kg/hr (max 1-2mEq/kg/hr)
- 3) Thiamine: up to 300 mg/day in 4 divided doses.

- 4) Riboflavin: 100 mg/day in 4 divided doses.
- 5) Add co-enzyme Q: 5-15 mg/kg/day
- 6) L-carnitine: 50-100 mg/kg orally.
- 7) Biotin 10 mg/day. (Biotin responsive Multiple carboxylase deficiency may present with unexplained lactic acidosis)¹²

Treatment of newborn with refractory seizures with no obvious etiology (suspected metabolic etiology)^{4, 13}

- 1) If patient persists to have seizures despite 2 or 3 antiepileptic drugs in adequate doses, consider trial of pyridoxine 100 mg intravenously. If intravenous preparation not available, oral pyridoxine can be given (15 mg/kg/day).
- 2) If seizures persist despite pyridoxine, give trial of biotin 10 mg/day and folinic acid 5mg twice daily (folinic acid responsive seizures). Trial of pyridoxal phosphate 10 mg/kg/dose X 2 doses is also recommended, however this is not available in India.
- 3) Rule out glucose transporter defect: measure CSF and blood glucose. In glucose transporter defect, CSF glucose level is equal to or less than 1/3rd of the blood glucose level. This disorder responds to the ketogenic diet.

Management of asymptomatic newborn with a history of sibling death with suspected IEM:

- 1) After baseline metabolic screen, start oral dextrose feeds (10% dextrose).
- 2) After 24 hours, repeat screen. If normal, start breast feeds. Monitor sugar, blood gases and urine ketones, blood ammonia 6 hourly.
- 3) Some authorities recommend starting medium chain triglycerides (MCT oil) before starting breast feeds,³ however, this is not being followed in our center (because of unpalatability of MCT oil).
- 4) After 48 hours, repeat metabolic screen. Obtain samples for TMS and urine organic acid tests.
- 5) The infant will need careful observation and follow-up for the first few months, as IEM may present in different age groups in members of the same family.

Long term treatment of IEM

The following modalities are available:

1) *Dietary treatment:* This is the mainstay of treatment in phenylketonuria, maple syrup urine disease, homocystinuria, galactosemia, and glycogen storage disease Type I & III. Special diets for PKU and MSUD are commercially available in the west. These are not available in India, but can be imported. These special diets are however very expensive, and cannot be afforded by most Indian patients. Based on the amino acid content of some common food products available in India, dietary exchanges are calculated and a low phenylalanine diet for PKU and diet low in branched chain amino acids for MSUD are being used in our center. However, there are no studies to document the efficacy of these

indigenous diets. Some disorders like urea cycle disorders and organic acidurias require dietary modification (protein restriction) in addition to other modalities.¹ The ketogenic diet is the treatment of choice for glucose transporter 1 deficiency and pyruvate dehydrogenase deficiency.

2) *Enzyme replacement therapy (ERT)*: ERT is now commercially available for some lysosomal storage disorders.¹⁵ However, these disorders do not manifest in the newborn period, an exception being Pompe's disease (Glycogen storage disorder Type II) which may present in the newborn period and for which ERT is now available.

3) *Cofactor replacement therapy*: The catalytic properties of many enzymes depend on the participation of non protein prosthetic groups, such as vitamins and minerals, as obligatory cofactors. The following co-factors may be beneficial in certain IEM: ¹⁶

- Thiamine: mitochondrial disorders, thiamine responsive variants of MSUD, PDH deficiency & complex I deficiency)
- Riboflavin: Glutaric aciduria Type I, Type II, mild variants of ETF, ETF-DH, complex I deficiency
- Pyridoxine: 50% of cases of homocystinuria due to cystathionine β -synthetase deficiency, pyridoxine dependency with seizures, xanthurenic aciduria, primary hyperoxaluria type I, Hyperornithemia with gyrate atrophy
- Cobalamin: Methylmalonic academia (*cblA*, *cblB*), Homocystinuria and methylmalonic academia (*cblC*, *cblD*, *cblF*)
- Folinic acid: Hereditary orotic aciduria, Methionine synthase deficiency, Cerebral folate transporter deficiency, hereditary folate malabsorption, Kearns-Sayre syndrome
- Biotin: Biotinidase deficiency, holocarboxylase synthetase deficiency

Prevention

1) **Genetic counselling and prenatal diagnosis**: Most of the IEM are single gene defects, inherited in an autosomal recessive manner, with a 25% recurrence risk. Therefore when the diagnosis is known and confirmed in the index case, prenatal diagnosis can be offered, wherever available for the subsequent pregnancies. The samples required are chorionic villus tissue or amniotic fluid. Modalities available are:¹⁷

- Substrate or metabolite detection: useful in phenylketonuria, peroxisomal defects.
- Enzyme assay: useful in lysosomal storage disorders like Niemann-Pick disease, Gaucher disease.
- DNA based (molecular) diagnosis: Detection of mutation in proband/ carrier parents is a prerequisite.

2) **Neonatal screening**: Tandem mass spectrometry is used in some countries for neonatal screening for IEM. Disorders which can be detected by TMS include

aminoacidopathies (phenylketonuria, MSUD, Homocystinuria, Citrullinemia, Argininosuccinic aciduria, hepatorenal tyrosinemia), fatty acid oxidation defects, organic acidemias (glutaric aciduria, propionic acidemia, methylmalonic acidemia, isovaleric acidemia). The appropriate time for collection of samples is between 1 and 3 days of life. The cost of this procedure is high, a potent disincentive for resource poor countries like India. Also, though the test is highly sensitive, the specificity is relatively low; and there are difficulties in interpretation of abnormal test results in apparently healthy infants.

Conclusion

IEM collectively represent an important cause of morbidity and death in the neonatal period. The most important step in the diagnosis of IEM is clinical suspicion. Screening investigations such as arterial blood gases, blood ammonia, lactate, sugar and urine ketones help to categorize the possible IEM and guide immediate management. Appropriate immediate treatment not only improves survival but also reduces the chances of neurodevelopmental sequelae.

Do's and Don't's

- 1) Don't think of IEM as rare, exotic and untreatable disorders.
- 2) Think of IEM in sick newborns in parallel with other common conditions such as sepsis. The signs and symptoms are usually non specific such as lethargy, poor feeding and vomiting.
- 3) Observe the precautions as explained while collecting and storing samples
- 4) Even if the chances of survival appear bleak, every attempt must be made to reach a diagnosis so that parents can be guided for future pregnancy.

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Table 1: Clinical pointers towards specific IEM's

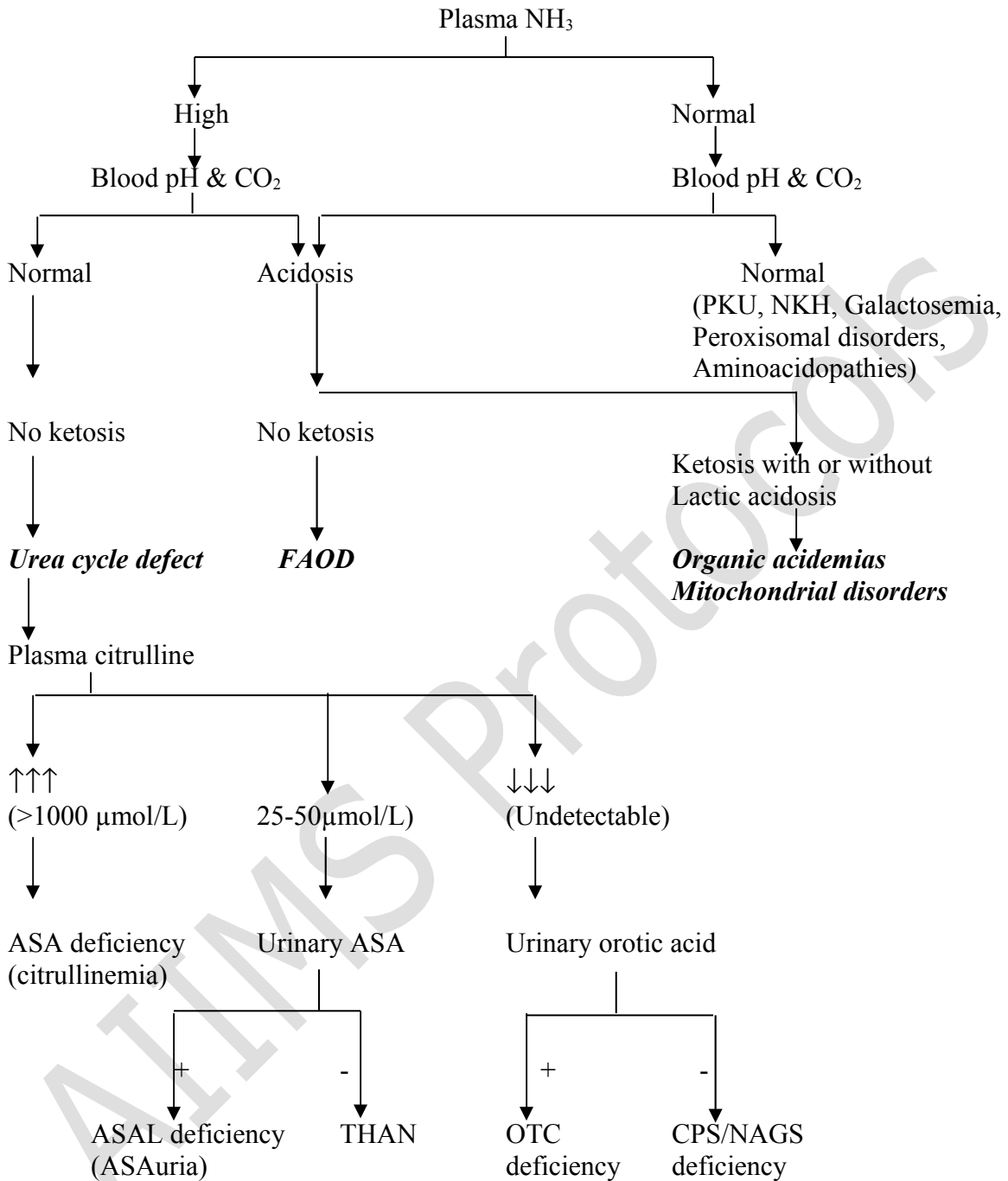
Clinical finding	Disorder
Coarse facies	Lysosomal disorders
Cataract	Galactosemia, Zellweger syndrome
Retinitis pigmentosa	Mitochondrial disorders
Cherry red spot	Lipidosis
Hepatomegaly	Storage disorders, urea cycle defects
Renal enlargement	Zellweger syndrome
Eczema/alopecia	Biotinidase deficiency
Abnormal kinky hair	Menke disease
Decreased pigmentation	Phenylketonuria
Hiccups	Nonketotic hyperglycinemia
Ophthalmoplegias	MSUD, Nonketotic hyperglycinemia

Table 2: Categorization of neonatal IEM using metabolic screening tests

Acidosis	Ketosis	Lactate	Ammonia	Diagnosis
-	+	-	-	Maple syrup urine disease
+	+/-	-	+/-	Organic aciduria
+	+/-	+	-	Lactic acidosis
-	-	-	+	Urea cycle
-	-	-	-	Non-ketotic hyperglycinemia , sulfite oxidase deficiency, peroxisomal, Phenylketonuria, galactosemia

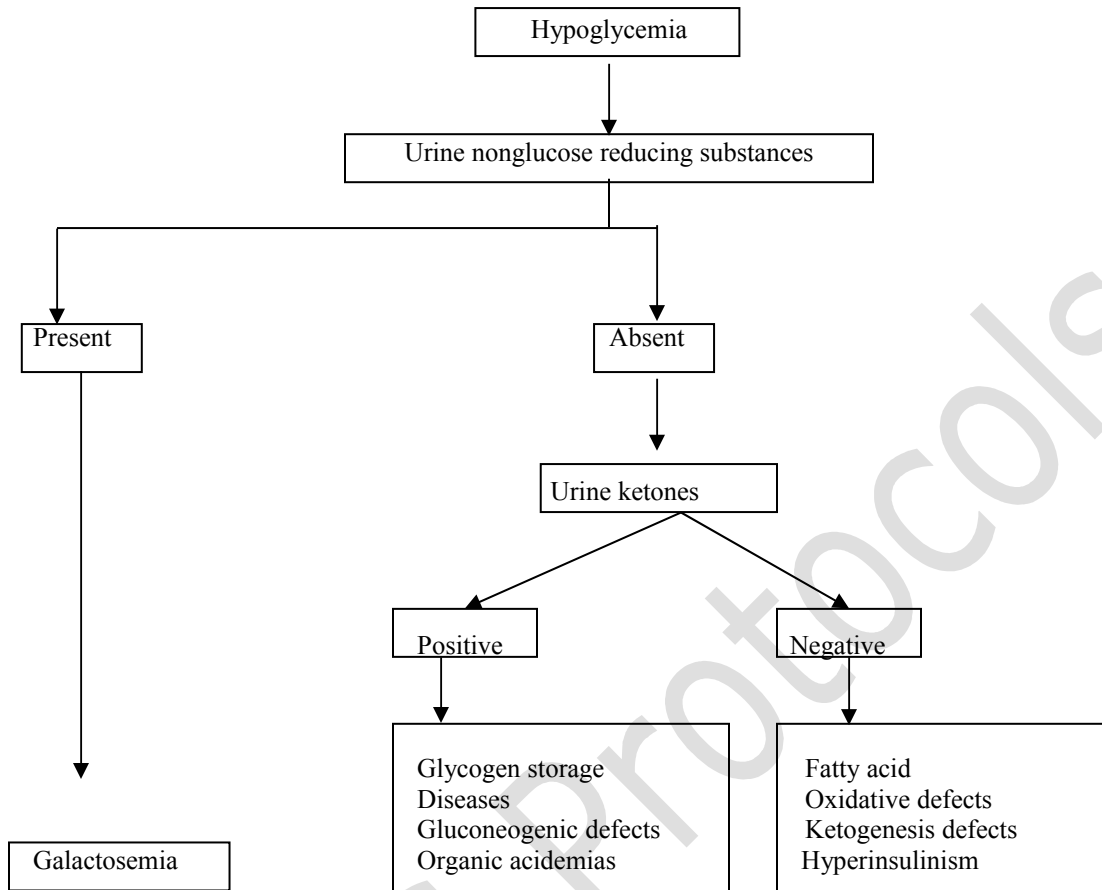
Fig 1: Approach to newborn with suspected metabolic disorder
Suspected Metabolic Disorder





(Abbreviations: FAOD: fatty acid oxidation defects, PKU: Phenylketonuria, NKH: Nonketotic hyperglycinemia, ASA: Argininosuccinic acid, OTC: Ornithine transcarbamoylase, CPS: carbamoyl phosphate synthetase I; NAGS: N-acetylglutamate synthetase, THAN: transient hyperammonemia of newborn, ASAL: argininosuccinic acid lyase)

Figure 2: Approach to newborn with persistent hypoglycemia and suspected IEM



Appendix: Commercially available formulations used in IEM

Co-factor	Trade name, formulation
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Pyridoxine	Tab Benadon (40mg) (Nicholas Piramal), Inj Vitneurin (1 ampoule contains 50 mg pyridoxine)
Hydroxycobalamin (Vitamin B12)	Inj Trineurosol (1000mcg/ml) (Tridoss Laboratories)
Thiamine	Tab Benalgis (75 mg) (Franco India)
Riboflavin	Tab Riboflavin (5 mg) (Shreya)
Biotin	Tab Essvit (5mg, 10mg) (Ecopharma)
Carnitine	Syrup L-Carnitor (5ml=500 mg), Tab L- Carnitor (500 mg), Inj carnitor (1g/5ml) (Elder)
Folinic acid	Tab Leukorin (15 mg) (Samrath)
Sodium Benzoate	Sachet 20g (Hesh Co.)
Arginine	ARG-9 Sachet (3g) (Noveau Medicament)
Coenzyme Q	Tab CoQ 30 mg, 50 mg. (Universal Medicare)