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INBORN ERRORS OF METABOLISM

EVALUATION AND MANAGEMENT OF A SICK INFANT WITH SUSPECTED INBORN ERROR OF METABOLISM

*Ratnakumari TL

Abstract: Inborn Errors of Metabolism (IEM) are not very uncommon. They present as great mimics to common diseases of children with symptoms such as tachypnea, apnea, convulsions and dehydration. In the newborn they mimic sepsis with non specific symptoms and most often the presentation can be acute and catastrophic, referred to as ‘metabolic distress’. Even though hundreds of IEM are described, making a diagnosis has been made simpler with advanced diagnostic tools like Tandem Mass Spectrometry (TMS) and genetic mutation studies which are currently available in India. With a ‘staged evaluation’ a diagnosis can be made and some of them can be treated effectively. This article is an attempt at giving a basic diagnostic approach for the management of a sick child, suspected to have IEM.

Keywords: IEM, Staged evaluation, Metabolic distress, Heritable disorder

The last two to three decades have generated a seemingly explosive interest in the field of “Inborn Errors of Metabolism” that, it becomes absolutely essential for the practicing pediatrician to know the basics of IEM. The ways of making a diagnosis of the same with a structured and objective approach should be the endeavour of every practitioner when he / she meets with a difficult neonate or child.

Though it is impossible for anyone to remember the specific symptoms or the complexities of every disorder under the realm of IEM, there lies a logic which forms the basic thread or network1 and by holding on to that thread one can make a reasonable attempt at a diagnostic approach. The following basic characteristics help in making such an approach and one must keep the following points in mind while setting out to workup a suspected IEM.

1. There are some disorders in which the errors in the biochemical pathway may affect only one functional organ or anatomic system and symptoms are exclusive to that system.

2. In others, the basic biochemical pathway may affect many systems in the body and hence the presenting symptoms are diverse, yet there could be an attempt at categorizing the diverse signs or symptoms.2

3. Biochemical abnormality may be a defect in intracellular trafficking.

When we come to the type of presentation it can be any one of the three types, a) intoxication type, b) energy deficiency type and c) storage type. It can be explained by going through the basic biochemistry with the following illustration (Fig.1).3

When a precursor “A” in the body is to be converted to product “C” through product “B”
with one helping enzyme in the pathway, the inadequacy or absence of that particular enzyme (E) results in one of three things -

1. Absence of product “C”
2. Excess of substance “A” and “B”
3. A new pathway taken by product “B” to produce new products “D” and “D1”

The result is, there is a deficiency of product “C”, an excess of products “A” and “B” and new products “D” and “D1” which are probably not needed for the system and can be toxic.

With this illustration, we can understand that the clinical signs and symptoms depend upon the function of that particular protein / enzyme of that particular pathway. The clinical presentation also depends upon whether the defect is confined to one physiologic system or has a multivariate role. If any one of the products cannot be degraded by the absence of a degrading enzyme, a) it will be stored (eg. Lysosomal storage disorders) or b) it can be toxic (eg. PKU) or c) if the product is absolutely essential for energy production it will also result in energy deficient state (eg. Mitochondrial disorders).

**Age of onset**

The signs and symptoms can manifest at any age from neonatal period through infancy and childhood to adulthood. Age of onset has a bearing since many IEM can have a typical age of onset. This is because the age of onset depends upon the developmental stage of a particular organ system, eg.cholesterol, peroxisomal biogenic disorders and lysosomal disorders can present at birth. The presentation may be insidious, eg.lysosomal storage disorders or acute, eg.peroxisomal disorders.

The IEM which involves intermediary metabolism presents after introduction of feeds, which can be acute and catastrophic referred to as “metabolic distress”. Even though the essential intermediary system is involved, we do not see fetal onset in such cases because of the fact that protein, carbohydrate and fat handling by the fetus is limited and the mother’s system handles it. This may not be true in disorders of large molecules which cannot be degraded by the system and they present at birth, eg.lysosomal storage disorders and peroxisomal disorders.

**IEMs affecting mother and fetus**

Some maternal diseases of IEM can affect the fetus, eg.PKU in the mother can cause dysmorphology in the fetus which results in congenital malformations (Fig.2). Vice versa, some disorders of the fetus can affect the mother too, eg.Very long chain hydroxy acyl CoA deficiency (VLCHAD) of fetus can manifest as acute fatty necrosis of maternal liver and hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome in the mother when she is...
heterozygous for VLCHAD.\textsuperscript{5} Similarly Medium chain acyl CoA deficiency (MCAD) and carnitine palmitoyl acyl coA transferase can cause acute fatty liver of pregnancy (AFLP).

Note:

- Energy deficiency disorders manifest within the first 24 hours of birth
- Neurodegenerative disorders manifest in infancy and childhood, eg. Canavans disease
- Porphyrias can manifest at infancy, childhood, adolescence and adult as neuropsychiatric illness

Having said all this, six periods of specific onset of presentation can be considered: at birth, 1 day to 1 month (neonatal), 1 month to 12 months (early infancy), 1 to 5 years (late infancy and early childhood), 5 to 15 years (late childhood and adolescence).

**Three types of onset**

- Acute stormy onset with rapid life threatening deterioration over hours, eg.some of the mitochondrial disorders with lactic acidosis.
- Episodic with intermittent decompensation and asymptomatic intervals, eg. some of the organic acidurias.
- Insidious onset with slow degeneration over decades, eg.neurodegenerative disorders - Canavan’s disease.

**Provocative circumstances**

Next step to be considered is ‘what exactly did provoke the symptoms in the child who was well earlier’. (Table 1)

**Symptoms and signs**

Symptoms and signs are myriad. Hence ‘when and how to suspect IEM’ always lies in the hands of the primary physician and it encompasses all that is said initially starting with conception. So suspect IEM when there is:\textsuperscript{6-9}

- Family history of fetal wastages, hydrops, severe IUGR, neonatal deaths and SIDS – not only for that family but for uncles, aunts both paternal and maternal and cousins. Negative family history does not rule out IEM.
- Coarse features, dysmorphism
- Unexplained clinical deterioration following a period of normalcy
- Unexplained odor (abnormal urine odor can be detected on a dry filter paper or by suddenly opening the lid of the container of stored urine at room temperature (Table.2).
- Feeding problems
- Persistent hiccoughs, change in tone, convulsions (hiccoughs when the child is well is not abnormal)
- Abnormal neurological signs
- Persistent tachypnoea, respiratory distress, apnoea
- Persistent lethargy
- Poor feeding, vomiting, diarrhea, and dehydration.
- Temperature instability.
- Unexplained shock
- Abnormal visceromegaly
- Acute Reye like syndrome
- Unexplained cardiomegaly with decompensation
- Cholestatic jaundice, (not biliary)
- IEM mimic sepsis in the newborn. (Also certain IEMs are associated with risk of sepsis, eg. galactosemia, CAH, organic acidemias)
- Abnormal acute laboratory abnormalities (biochemical and hematological)
Table 1. Most frequent provocative circumstances observed in inborn errors of intermediary metabolism

<table>
<thead>
<tr>
<th>Symptoms triggered by:</th>
<th>Carbohydrate</th>
<th>Catabolic circumstances</th>
<th>Infection</th>
<th>Fever, fasting</th>
<th>Surgery</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weaning</strong></td>
<td>* Fructose intolerance, Fructose</td>
<td>* Aminoacidopathies</td>
<td>* Organic acidurias</td>
<td>* Fatty acid oxidation disorders, Urea cycle defects, Gluconeogenesis defects, Glycogenosis defects</td>
<td>* All conditions listed above</td>
<td>* Porphyria, Glucose-6-phosphate dehydrogenase deficiency</td>
</tr>
<tr>
<td></td>
<td>diphosphatase deficiency, Urea cycle defects, Lysinuric protein intolerance, Triple-H syndrome, Maple syrup urine disease, Organic acidurias</td>
<td></td>
<td></td>
<td>* Thromboembolic accident in homocystinuria</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fructose</strong></td>
<td>* Fructose intolerance, Fructose</td>
<td></td>
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<tr>
<td></td>
<td>diphosphatase deficiency</td>
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<td><strong>Galactose</strong></td>
<td>* Galactosemia</td>
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<tr>
<td><strong>Glycerol</strong></td>
<td>* Glycerol intolerance</td>
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<tr>
<td><strong>Protein</strong></td>
<td>* Urea cycle defects, Lysinuric protein intolerance, Triple-H syndrome, Maple syrup urine disease, Organic acidurias, Hyperinsulinism (with hyperammonemia)</td>
<td></td>
<td></td>
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Table 2. List of IEM with unusual odours

<table>
<thead>
<tr>
<th>IEM</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isovaleric aciduria</td>
<td>Sweaty feet</td>
</tr>
<tr>
<td>Maple syrup urine disease</td>
<td>Maple syrup</td>
</tr>
<tr>
<td>Phenyl ketonuria</td>
<td>Musty</td>
</tr>
<tr>
<td>Tyrosinemia</td>
<td>Cabbage</td>
</tr>
<tr>
<td>Glutaric aciduria type II</td>
<td>Sweaty feet</td>
</tr>
<tr>
<td>Multiple carboxylase</td>
<td>Male cat urine</td>
</tr>
</tbody>
</table>

like: Persistent hypoglycemia, persistent acidosis, persistent abnormal hepatic and renal parameters, hyperammonemia, persistent abnormal coagulation profile, pancytopenia, leucopenia, thrombocytopenia.

- Hyperammonemia, ketosis, abnormal amino acid profile and positive DNPH test in the absence of glycosuria and acetonuria.

**Clinical findings in children and adolescence**

When it comes to children and adolescents, failure to thrive, more expressive neurological signs and developmental delay will be remarkable. Dysmorphic features get more
grotesque and systemic signs start appearing, eg.1 In Morquo’s syndrome the dysmorphic features express themselves as age advances, eg.2 In homocystinuria, molybdenum cofactor defect, sulfite oxidase deficiency, dislocation of ocular lens appears after 2 to 3 months of age.\textsuperscript{10}

- One always has to look for ocular, skin, hematologic, visceral and endocrine symptoms and findings.

- Acute symptoms may be precipitated by minor viral infections.

Before starting with the ‘work up’ to evaluate an infant with suspected IEM, we will consider the following simple lessons.

\textbf{Lesson 1. IEM involving protein metabolism / aminoacidopathies}\textsuperscript{1}

Aminoacids (AAs) are made up of amino group and an organic acid backbone. When there is a block in their metabolic pathway, two major findings arise - a) since both amino radical and acid component can not be degraded, there is acidosis as well as hyperammonemia (when urea cycle is overwhelmed) b) the particular aminoacid can not contribute to the pool of neoglucogenesis and hence, there is hypoglycemia. The same is true for all aminoacids when the metabolic defect is in the intermediary pathway. So to recapitulate, in organic acidemias there is acidosis (usually not lactic), hypoglycemia and there could be hyperammonemia since some of the by-products inhibit urea cycle, eg. propionic acidemia. The by-products interfere with acetyl coA synthesis and hence lactic acidosis may be there. (Fig.3)

For aminoacids which do not take part in intermediary metabolism the symptoms are mostly toxic, eg. PKU

\textbf{Lesson 2. IEM involving carbohydrates}

There are three points for consideration

a) Hypoglycemia can result from glycogen storage disorders, glycolytic pathway disorders, aminoacidopathies, citric acid cycle disorders, neoglucogenic disorders, beta oxidation of fat disorders and may accompany mitochondrial disorders. (Hyper insulimemic state, a separate entity, is not dealt here)

b) Pure glucose is not the only carbohydrate source. Diet consists of the following sugars, namely fructose, sucrose, lactose and galactose which have to be converted to glucose to enter into oxidative phosphorylation (Fig.4). Failure of conversion can lead to 1. accumulation of toxic metabolites and 2. hypoglycemia

c) Glycogen storage disorder\textsuperscript{12} (Fig.5): The storage product of carbohydrate metabolism is glycogen. In times of need, stored glycogen is to be broken down to glucose with the help of enzymes. If it can not be broken down, it will be stored and there will be visceromegaly with hypoglycemia and usually ketosis (one exception is type I glycogenosis).

\textbf{Lesson 3. Urea cycle disorders}

In urea cycle disorders ammonia cannot be converted to urea and hence urea levels can be low and ammonia levels are grossly elevated.
(Fig.6). Usually there is no hypoglycemia. There is tachypnoea because ammonia is a potent respiratory centre stimulent and the sign is often mistaken for acidosis.

**Lesson 4. Respiratory chain / mitochondrial disorders**

Glucose is the energy currency for human organism. But at the cellular level ATP is the currency of energy. Stored chemical energy from glucose has to be transferred to ATP through several steps. In the final step, failure of conversion of NADH to ATP leads to accumulation of NADH and in turn lactic acid, because increased NADH favors lactic acid (pyruvate and lactic acid are in balancing equilibrium) (Fig.7).

**Lesson 5. Beta oxidation of fat**

In the metabolism of fat, degradation starts with splitting of fat into free fatty acids and glycerol. Free fatty acid enters into the cell along with carnitine and subsequent beta oxidation takes place within the mitochondria with production of ketone bodies. In defects of beta oxidation there is acidosis (fatty acid accumulation) and hypoglycemia because of ineffective contribution to neoglucogenic pool, and there is striking absence of ketone bodies. Since urea cycle is inhibited by the by-products, hyper-ammonemia can be there, but not severe. There is secondary carnitine deficiency with excessive excretion of acyl carnitine.

**Lesson 6. Cholesterol synthesis defects**

In cholesterol synthesis defects, there could be dysmorphism with hypoglycemia since the precursors for cholesterol are aminoacids.

**Lesson 7. Storage disorders**

In storage disorders there will be hepatosplenomegaly and cardiomegaly with dysmorphism.
Investigations

With the above background the investigatory guidelines are given as below:
1. Start with routine simple tests
2. Then proceed with specific tests
3. Special tests when circumstances warrant

The Table.3 gives categories of tests to be done. Physician should be judicious in choosing them and be guided by the stems given in the text.

After the basic and some of the specific investigations are done, at least one of the following findings are observed in most of the IEMs except in certain neurodegenerative disorders like non ketotic hyperglycinemia, sulfite oxidase defect or Smith Lemli opitz syndrome. They are,

1. Hypoglycemia
2. Acidosis
3. Hyper ammonemia
4. Ketosis (when concentration of ketone bodies are more than 7mM/l)
5. Positive / negative DNPH tests
6. Lactic acidosis

Holding on to any one stem can lead on to the diagnosis (Figs. 8, 9, 10, 11, 12, 13)
### Table 3. Initial investigations

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Basic investigations</th>
<th>Specific investigations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>Smell (special odor) Look (special color) Acetone (Acetest) Reducing substances (Clinitest) Keto acids (DNPH) pH (pHstix) Sulfitest Electrolytes (Na+, K+) Uric acid (search for hypouricuria)</td>
<td>Urine collection: collect each fresh micturition separately and put it in the refrigerator. Freezing: freeze at -20°C samples collected before treatment and, afterward, an aliquot of 24-h collection on treatment. Do not use for specific investigations without expert metabolic advice.</td>
</tr>
<tr>
<td>Blood</td>
<td>Complete blood count Electrolytes (search for anion gap) Glucose, calcium Blood gases (pH, ( \text{PCO}_2 ), ( \text{HCO}_3^- ), ( \text{PO}_2 )) Uric acid Prothrombin time Transaminases (and other liver tests) Ammonemia Lactic and pyuvic acids ( \alpha )-hydroxybutyrate, acetoacetate</td>
<td>Plasma, heparinized, 5 ml at -20°C Blood on filter paper (as “Guthrie test”) Whole blood 10 to 15 ml collected on EDTA and frozen (for molecular biology studies)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Lumbar puncture Chest x-ray Echocardiography, EKG Cerebral ultrasound, EEG Free fatty acids</td>
<td>Skin biopsy (fibroblast culture) Cerebrospinal fluid, 1 ml frozen Postmortem: liver, muscle biopsies (macroscopic fragment frozen at -70°C)</td>
</tr>
</tbody>
</table>

A Stage evaluation always helps

![Diagram of screening, specific, and specialised tests](image-url)
When persistent hypoglycemia is encountered, the following stem can be utilised (Hyper insulinemic syndromes are omitted)

Fig. 8 Stem 1 - Hypoglycemia\textsuperscript{15}
The other approach to hypoglycemia is to do DNPH, ABG and estimation of ketone bodies and then proceed with the stem.

Fig. 9 Stem 2 - Hypoglycemia$^{15}$
Lactic acidemia is an important feature of respiratory chain and Kerbs cycle disorders.
CPS - Carbamoyl Phosphate synthetase
OTC - Ornithine trans carbamylase
ASL - Arginino succinic acid lyase
ASS - Arginino succinic acid synthase

Fig.13 Stem 6 - Urea cycle defect

The above said stems are over simplification of metabolic pathways and are shown just to make one understand, how to approach a child with an IEM, involving the intermediary metabolism. The exact pathway, enzymes, etc are willfully omitted. Single metabolic pathways not involving the energy generation like, bile acid metabolism of porphyrin metabolism are to be worked up as per need.

The reader should understand that this topic does not cover all the IEM. It covers only simple intermediary metabolic defects and one can refer to the list of books given at the end of this article.

The acute management protocol for a sick child with suspected IEM is given below for guidance.

Acute management of sick child with suspected IEM\textsuperscript{13,14}

In the management of inborn error of metabolism, stabilisation is the hallmark.

Whatever be the IEM, supportive care should be started at the emergency room itself.

I. General

1. Supportive

Take care of ABC.

Correct hypothermia, hypoglycemia and dehydration

Start arterial and central venous access

Give respiratory support, if needed.
2. **Antibiotics**  
Start on broad spectrum antibiotics along with metronidazole and neomycin to control the possibility of production of organic acids by intestinal bacteria

3. **Shock**  
Correct shock with fluid boluses 10-20 ml/per kg of normal saline, repeat as per protocol  
Avoid Ringer Lactate (RL) if acidosis is already there  
Avoid hypotonic fluid to prevent cerebral edema

4. **Prevention of cerebral edema**  
Restrict fluids, if signs of cerebral edema start to appear  
If cerebral edema, give IV Mannitol 0.25-0.5g/kg and IV frusemide 1mg/kg.

5. **Stop all proteins**  
Stop all proteins for 48 to 72 hrs including TPN if the child as on TPN

6. **Avoid offending sugars**  
Stop offending agents like galactose and fructose if the disorder is known

7. **Treat hypoglycemia**  
With IV glucose 2ml/kg (200-400 mg/kg) of D20 / D25 if there is central line

8. **Fluid volume and glucose infusion rate**  
Maintain with D10 with electrolytes as needed at 1-1.5 times maintenance volume  
Keep glucose infusion rate (GIR) as 8-10 mg/kg/min at 1-1.5 times maintenance GIR  
To prevent protein catabolism, give 50 to 70 kcal / kg / 24hrs (as above)  
Keep blood glucose levels at 120 to 170 mg/dL

9. **Promote anabolism**  
Give IV Insulin 0.05 to 0.1 U per kg / hr upto 0.2 to 0.3 U to further promote anabolism

10. **Correct acidosis (pH <7 to 7.2)**  
Correct metabolic acidosis slowly and cautiously with IV sodium bicarbonate, give 0.35 to 0.5 mEq of sodium bicarbonate upto 1mEq/kg/hr or potassium acetate 1mEq/kg/hr through IV infusion.  
Start hemodialysis if intractable acidosis is encountered (Peritoneal dialysis less effective).

11. **Low protein**  
When oral feeds are started after stabilization, give low protein diet (0.7 g/kg/per 24 hrs).

II. **Elimination of toxic metabolites (if disorder is suspected or known)**

1. **For hyperammonemia**  
For OTC and CPS deficiency, start on priming dose of sodium phenylacetate 250 mg/kg and Sodium benzoate 250mg/kg as “ammonia trap” [(ammonul 2.5 ml) (‘ammonul’ contains sodium benzoate 100 mg and sodium phenylacetate 100mg/mL)] along with, arginine HCl 200 mg/kg in 25 ml/kg of 10% dextrose solution over 90 minutes. This is followed by maintenance dose of all the three drugs; 250 mg of sodium benzoate and sodium phenylacetate each and 200 mg of arginine / day (oral).

**Priming dose for Ammonia trapping**

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<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium phenylacetate</td>
<td>- 250 mg/kg</td>
</tr>
<tr>
<td>Sodium Benzoate</td>
<td>- 250 mg/kg</td>
</tr>
<tr>
<td>Arginine Hydrochloride</td>
<td>- 200 mg/kg</td>
</tr>
</tbody>
</table>

Followed by same dose of all drugs every day orally
For citrullinemia and arginino succinic academia, give same drugs except increase L-arginine HCl in a dose of 600 mg/kg for loading and sustained infusion.

For argininase deficiency, use same regimen of ‘ammonul’ for loading and sustained infusion, but omit L-arginine HCl.

If neonate/infant is not critically ill and hyperammonemia is mild, arginine therapy alone may suffice.

For all urea cycle disorders, IV therapy of ammonia scavenging drugs to be continued, while dialysis is being performed. A repeat loading dose of ammonia-scavenging drugs should be given only in neonates with very severe illness who are receiving dialysis. Toxicity is associated with high drug doses (750 mg/kg/day and higher). Hemodialysis to be done when NH3 > 500 micro mol/l

If the disorder is organic acidemia, give carnitine 100mg/kg/24hrs IV (or) oral glycine 250 mg/kg/24hrs (mainly for isovaleric acidemia) as diversion therapy

For presumed carnitine deficiency and life threatening amino acidopathies, administer cofactor IV L-carnitine (25 to 100 mg/kg/day IV).

Carnitine therapy is controversial if the disorder is fatty acid oxidation defect

Hydration promotes renal excretion of toxins.

III. Stimulation of residual enzyme activity with high dose of co-factors

Pyridoxine (B6) 100 mg IV for pyridoxine dependency. Maintain with 2 to 10mg / day

For folic acid responsive seizures 10-20mg of folic acid.

If the disorder is known

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl malonic acidemia</td>
<td>Vit B12 1mg IM/day</td>
</tr>
<tr>
<td>Biotinidase deficiency</td>
<td>Biotin 10 to 60 mg/day oral</td>
</tr>
<tr>
<td>Multiple carboxylase deficiency</td>
<td>Biotin</td>
</tr>
<tr>
<td>Glutaric aciduria - type II</td>
<td>Riboflavin 100 to 300 mg/ day oral</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>Pyridoxine IM/IV 200 to 1000mg/24hrs</td>
</tr>
<tr>
<td>Carnitine deficiency</td>
<td>Carnitine 100 to 400 mg of L-carnitine/kg/day oral</td>
</tr>
<tr>
<td>MSUD (Maple syrup urine disease)</td>
<td>Thiamine 10mg to 200mg/24hrs Riboflavin 200 to 300 mg/oral TID</td>
</tr>
<tr>
<td>Mevalonic acidemia</td>
<td>Prednisone 2mg / kg / 24 hrs</td>
</tr>
<tr>
<td>Hartnup disease</td>
<td>Nicotinamide 50 to 300mg/day High protein</td>
</tr>
<tr>
<td></td>
<td>Chronic treatment includes a special diet small amount of offending aminoacids and related metabolites</td>
</tr>
<tr>
<td></td>
<td>To sum up, contrary to the myth, the reality is, IEM are quite common in the scenario of over populous nation like India and some of them are effectively treatable.</td>
</tr>
</tbody>
</table>

To sum up, contrary to the myth, the reality is, IEM are quite common in the scenario of over populous nation like India and some of them are effectively treatable.
Points to Remember

- **IEM are not uncommon**
- **Do suspect IEMs in all babies with unexplained deterioration of clinical condition and suspected sepsis when sepsis screen is negative.**
- **Start with a simple approach to hold on to a ‘thread of logic’ which will lead on to the diagnosis.**
- **Stabilization is the key to management.**
- **If a diagnosis is not made when the child is alive, do collect blood samples and freeze to send for subsequent analysis.**
- **An attempt to make a diagnosis gives the choice to the parents in subsequent pregnancies.**
- **We have a long way to go in effective treatment and in the current scenario genetic counseling to the parents is the crux.**

Bibliography

### Table 1 - Classification

**Errors of IEM in:**

- Aminoacid metabolism
- Carbohydrate metabolism
- FA oxidation
- Fat metabolism eg; storage disorders
- Respiratory chain eg; mitochondrial disorders
- Large molecule metabolism

1. Disorder of glycoconjugate biosynthesis
2. Lysosomal storage disorder
   - Small molecule metabolism
   1. Cholesterol biosynthetic defects
2. Organic acidurias
3. Peroxisomal disorders
   (a) Biogenesis defects
   (b) Single enzyme defects
   - Others: Bile acid metabolism
   - Hemoglobinopathies
   - Uric acid metabolism
   - Mineral metabolism
   - Cofactor (Vitamin) metabolism
   - Hyperlipidemias
<table>
<thead>
<tr>
<th>Finding</th>
<th>Diagnostic Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidosis</td>
<td>Fatty acid oxidation defects</td>
</tr>
<tr>
<td></td>
<td>Gluconeogenesis defects</td>
</tr>
<tr>
<td></td>
<td>Glycogen storage diseases</td>
</tr>
<tr>
<td></td>
<td>Ketogenesis defects</td>
</tr>
<tr>
<td></td>
<td>Ketolysis defects</td>
</tr>
<tr>
<td></td>
<td>Krebs cycle defects</td>
</tr>
<tr>
<td></td>
<td>Organic acidemias</td>
</tr>
<tr>
<td></td>
<td>Respiratory chain defects</td>
</tr>
<tr>
<td>Alkalosis</td>
<td>Urea cycle defects</td>
</tr>
<tr>
<td>Respiratory Metabolic</td>
<td>Steroid biosynthetic defects</td>
</tr>
<tr>
<td>Hepatic dysfunction</td>
<td>Amino acid defects</td>
</tr>
<tr>
<td></td>
<td>Bile acid biosynthesis defects</td>
</tr>
<tr>
<td></td>
<td>Carbohydrate defects</td>
</tr>
<tr>
<td></td>
<td>Fatty acid oxidation defects</td>
</tr>
<tr>
<td></td>
<td>Peroxisomal disorders</td>
</tr>
<tr>
<td></td>
<td>Respiratory chain defects</td>
</tr>
<tr>
<td></td>
<td>Other</td>
</tr>
<tr>
<td>Hyperammonemia</td>
<td>Amino acid disorders</td>
</tr>
<tr>
<td></td>
<td>Fatty acid oxidation defects</td>
</tr>
<tr>
<td></td>
<td>Organic acidemias</td>
</tr>
<tr>
<td></td>
<td>Urea cycle defects</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>Fatty acid oxidation defects</td>
</tr>
<tr>
<td></td>
<td>Gluconeogenesis defects</td>
</tr>
<tr>
<td></td>
<td>Glycogen storage disease</td>
</tr>
<tr>
<td></td>
<td>Ketogenesis defects</td>
</tr>
<tr>
<td></td>
<td>Organic acidemias</td>
</tr>
<tr>
<td>Ketosis/ketonuria</td>
<td>Amino acid defects</td>
</tr>
<tr>
<td></td>
<td>Gluconeogenesis defects</td>
</tr>
<tr>
<td></td>
<td>Glycogen storage disease</td>
</tr>
<tr>
<td></td>
<td>Ketogenesis defects</td>
</tr>
<tr>
<td></td>
<td>Organic acidemias</td>
</tr>
<tr>
<td>Pancytopenia</td>
<td>Organic acidemias</td>
</tr>
<tr>
<td></td>
<td>Respiratory chain defects</td>
</tr>
<tr>
<td>Proximal renal tubular dysfunction</td>
<td>Amino acid defects</td>
</tr>
<tr>
<td></td>
<td>Carbohydrate defects</td>
</tr>
<tr>
<td></td>
<td>Respiratory chain defects</td>
</tr>
</tbody>
</table>
Table 3. Characteristic urinary findings in inborn errors of metabolism\textsuperscript{2,5}

<table>
<thead>
<tr>
<th>Finding</th>
<th>Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing substance</td>
<td>Hereditary fructose intolerance</td>
</tr>
<tr>
<td></td>
<td>Galactosemia</td>
</tr>
<tr>
<td></td>
<td>Hereditary tyrosinemia</td>
</tr>
<tr>
<td>Acetonuria</td>
<td>Organic acidemias</td>
</tr>
<tr>
<td>DNP (-ketoacids)</td>
<td>Maple syrup urine disease</td>
</tr>
<tr>
<td></td>
<td>Phenylketonuria</td>
</tr>
<tr>
<td></td>
<td>Hereditary tyrosinemia</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>Phenylketonuria</td>
</tr>
<tr>
<td></td>
<td>Hereditary tyrosinemia</td>
</tr>
<tr>
<td>Nitrosonaphthol</td>
<td>Hereditary tyrosinemia</td>
</tr>
<tr>
<td>-Nitroaniline</td>
<td>Methylmalonic academia</td>
</tr>
<tr>
<td>Sulfitest</td>
<td>Sulfite oxidase deficiency</td>
</tr>
</tbody>
</table>

Table 4. Specialized laboratory tests that may be required for the care of neonates with suspected metabolic disease\textsuperscript{2,5}

<table>
<thead>
<tr>
<th>Body fluid or tissue</th>
<th>Laboratory tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Amino acids</td>
</tr>
<tr>
<td></td>
<td>Carnitine (total, free, and acylcarnitine profile)</td>
</tr>
<tr>
<td></td>
<td>Lactate and pyruvate</td>
</tr>
<tr>
<td></td>
<td>Very-long-chain fatty acids and phytanic acid (peroxisomal disorders)</td>
</tr>
<tr>
<td></td>
<td>Transferrin isoelectric focusing – CDG</td>
</tr>
<tr>
<td></td>
<td>Immuno-electrophoresis (carbohydrate-deficient glycoprotein syndromes)</td>
</tr>
<tr>
<td>Urine</td>
<td>Amino acids</td>
</tr>
<tr>
<td></td>
<td>Organic acids</td>
</tr>
<tr>
<td></td>
<td>Carnitine (total, free, and acylcarnitine profile)</td>
</tr>
<tr>
<td></td>
<td>Glycolipids,</td>
</tr>
<tr>
<td></td>
<td>oligosaccharides, and mucopolysaccharides</td>
</tr>
<tr>
<td></td>
<td>(Lysosomal storage disorders)</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>Amino acids</td>
</tr>
<tr>
<td>Other sources</td>
<td>Lactate and pyruvate</td>
</tr>
<tr>
<td>Cultured skin fibroblasts</td>
<td>Genetic studies</td>
</tr>
<tr>
<td>White cells</td>
<td>Genetic studies</td>
</tr>
</tbody>
</table>
Table 5. Neonatal-onset inborn errors of metabolism characterized by abnormal plasma amino acid patterns\textsuperscript{2, 5}

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid disorders</td>
<td></td>
</tr>
<tr>
<td>Maple syrup urine disease</td>
<td>↑ Isoleucine, leucine, valine</td>
</tr>
<tr>
<td>Nonketotic hyperglycinemia*</td>
<td>↑ Glycine</td>
</tr>
<tr>
<td>Phenylketonuria</td>
<td>↑ Phenylalanine</td>
</tr>
<tr>
<td>Sulfite oxidase deficiency</td>
<td>↑ S-Sulfocysteine</td>
</tr>
<tr>
<td>Hereditary tyrosinemia</td>
<td>↑ Tyrosine, methionine</td>
</tr>
<tr>
<td>Lactic acidemias</td>
<td>↑ Alanine and …</td>
</tr>
<tr>
<td>PC deficiency</td>
<td>↑ Citruline, lysine</td>
</tr>
<tr>
<td>Organic acidemias</td>
<td></td>
</tr>
<tr>
<td>Methylmalonic acidemia</td>
<td>↑ Glycine</td>
</tr>
<tr>
<td>Isovaleric acidemia</td>
<td>↑ Glycine</td>
</tr>
<tr>
<td>Propionic acidemia</td>
<td>↑ Glycine</td>
</tr>
<tr>
<td>Urea cycle defects</td>
<td>↑ Glutamine, ↓ arginine</td>
</tr>
<tr>
<td>Specific disorder</td>
<td></td>
</tr>
<tr>
<td>Argininosuccinic aciduria</td>
<td>↑ ASA, ↑ Citrulline</td>
</tr>
<tr>
<td>Citrullinemia</td>
<td>↑↑ Citrulline</td>
</tr>
<tr>
<td>CPS and OTC deficiency</td>
<td>↑ Citrulline</td>
</tr>
<tr>
<td>PC - Pyruvate carboxylase</td>
<td></td>
</tr>
<tr>
<td>CPS - Carbamyle phosphate synthatase</td>
<td></td>
</tr>
<tr>
<td>OTC - Ornithine trans carbamylase</td>
<td></td>
</tr>
<tr>
<td>ASAS - Arginino succinic acid synthetase</td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Prenatal diagnostic tests\textsuperscript{2, 5}

<table>
<thead>
<tr>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amniocentesis</td>
</tr>
<tr>
<td>Chorionic villus biopsy</td>
</tr>
<tr>
<td>Liver biopsy</td>
</tr>
<tr>
<td>Restriction Fragment Length Polymorphism (RFLP) – CPS, OTC, Arginase deficiency.</td>
</tr>
</tbody>
</table>
Table 7. Inborn Errors of Metabolism that cause dysmorphic syndromes²,⁵

<table>
<thead>
<tr>
<th>Inborn Error</th>
<th>Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Large-molecule metabolism</strong></td>
<td></td>
</tr>
<tr>
<td>Disorder of glycoconjugate biosynthesis</td>
<td>Carbohydrate-deficient glycoprotein syndromes</td>
</tr>
<tr>
<td>Lysosomal storage</td>
<td>Glycolipidoses</td>
</tr>
<tr>
<td>Diseases</td>
<td>Mucopolysaccharidoses</td>
</tr>
<tr>
<td></td>
<td>Oligosaccharidoses</td>
</tr>
<tr>
<td><strong>Small-molecule metabolism</strong></td>
<td></td>
</tr>
<tr>
<td>Cholesterol biosynthetic Defects</td>
<td>Conradi-Hunermann syndrome</td>
</tr>
<tr>
<td>Organic acidurias</td>
<td>Desmosterolosis Smith-Lemli-Opitz syndrome</td>
</tr>
<tr>
<td></td>
<td>3-Hydroxyisobutyryl deacylase deficiency</td>
</tr>
<tr>
<td></td>
<td>Mevalonic aciduria*</td>
</tr>
<tr>
<td></td>
<td>Multiple acyl-CoA dehydrogenase deficiency</td>
</tr>
<tr>
<td><strong>Peroxisomal disorders</strong></td>
<td></td>
</tr>
<tr>
<td>Biogenesis disorders</td>
<td>Chondrodysplasia punctata, rhizomelic type</td>
</tr>
<tr>
<td></td>
<td>Zellweger syndrome and its variants</td>
</tr>
<tr>
<td></td>
<td>Infantile Refsum disease</td>
</tr>
<tr>
<td></td>
<td>Neonatal adrenoleukodystrophy</td>
</tr>
<tr>
<td><strong>Single enzyme defects</strong></td>
<td></td>
</tr>
<tr>
<td>Defects of peroxisomal fatty</td>
<td>Acyl-CoA oxidase deficiency</td>
</tr>
<tr>
<td>acid oxidation</td>
<td>Bifunctional enzyme deficiency</td>
</tr>
<tr>
<td></td>
<td>Thiolase deficiency</td>
</tr>
</tbody>
</table>

Table 8. Disorders associated with abnormal liver function²,⁵

<table>
<thead>
<tr>
<th>Category of disorder</th>
<th>Defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid metabolism</td>
<td>Hereditary tyrosinemia</td>
</tr>
<tr>
<td>Carbohydrate metabolism</td>
<td>Galactosemia</td>
</tr>
<tr>
<td></td>
<td>Hereditary fructose intolerance</td>
</tr>
<tr>
<td>Fatty acid oxidation</td>
<td>Carnitine</td>
</tr>
<tr>
<td></td>
<td>Palmitolytransferase II deficiency</td>
</tr>
<tr>
<td></td>
<td>Long-chain 3-hydroxyacyl-CoA dehydrogenase Deficiency</td>
</tr>
<tr>
<td></td>
<td>Multiple acyl-CoA Dehydrogenase deficiency</td>
</tr>
<tr>
<td>Peroxisomal disorders</td>
<td>Neonatal adrenoleukodystrophy</td>
</tr>
<tr>
<td></td>
<td>Zellweger syndrome</td>
</tr>
<tr>
<td>Respiratory chain</td>
<td>Complex IV</td>
</tr>
<tr>
<td></td>
<td>mtDNA depletion syndrome</td>
</tr>
<tr>
<td>Other</td>
<td>á₁ Antitrypsin deficiency</td>
</tr>
<tr>
<td></td>
<td>Carbohydrate-deficient glycoprotein syndrome (CDG)</td>
</tr>
<tr>
<td></td>
<td>Glycogen storage disease type IV</td>
</tr>
<tr>
<td></td>
<td>Niemann-Pick disease type C</td>
</tr>
</tbody>
</table>
### Table. 9. IEMs with Hepatomegaly / Splenomegaly\(^2,^5\)

- GSDs
- Lipidoses - Farbers, Gauchers, Krabbe, Wolman diseases, GM1 gangliosidosis, Niemann-Picks disease
- Mucopolysaccharidoses
- Oligosaccharidoses - I cell disease, Sialidosis, Fucosidosis

### Table. 10. IEMs with Cardiomegaly / Cardiomyopathy\(^2,^5\)

- FA oxidation - Carnitine- acylcarnitine translocase def. Carnitine palmitoyl transferase def. LCHAD def. VLCHAD def.
- GSDs - type II and IX
- Lysosomal storaged diseases - glycolipidoses mucopolysaccharidoses oligosaccharidoses
- Respiratory chain defect
- CDG syndromes

### Table. 11. Investigations for suspected IEM if child dies without a diagnosis\(^2,^5\)

- 5 ml Red cells at +4\(^*\)c
- 5 ml Plasma at -20\(^*\)c
- 10-20 ml Urine at -20\(^*\)c
- Biopsy – skin (for fibroblast culture), Liver, Muscle, Brain

### Table. 12- Dietary tips\(^2,^5\)

- Fructose intolerance - avoid fruit juices
- Pyruvate dehydrogenase def. - high fat, low carbohydrate
- Organic acidemias - high carbohydrate, low protein and fat continuous feeding avoid fasting
- B-Oxidation defects - high carbohydrate, low fat diet
- Ketolysis defect - low fat, low protein carnitine supplementation
- LCHAD def. - medium Chain triglycerides
- Purine and pyridine metabolism - avoid nuts, non-veg.
Prenatal Diagnosis and Newborn Screening: Relevance in India

*Mamta Muranjan
**Shruti Agarwal

Abstract: Inborn errors of metabolism (IEM) are progressive disorders characterized by high fatality and permanent disability in survivors. They are growing at an alarming rate in India. Evaluation and treatment of these disorders is expensive and not easily available. In a small proportion of patients who have access to therapy, the outcome may not be optimum due to late diagnosis and therapy. The option for many families affected by these disorders is prenatal diagnosis and newborn screening. Prenatal diagnosis for IEM is available with non-invasive modalities such as ultrasonography and biochemical, histopathological or molecular testing of fetal tissues obtained by invasive procedures and is fairly well established for lysosomal storage disorders with enzyme estimation. However in many cases, diagnosis in the index case is not established. In such situations newborn screening is often advised for high risk screening. Universal newborn screening is not yet practised except in some isolated regions. The options for universal newborn screening for IEM in India and the hurdles to be overcome are discussed.

Keywords: Inborn errors of metabolism, Chorion villus sampling, Amniocentesis, Ultrasonography, Fetal MRI, Tandem Mass Spectroscopy

Inborn errors of metabolism (IEM) are biochemical disorders in which an abnormality of the enzyme or a transport protein can give rise to pathological consequences at birth or later in life. Although individually rare, as a group these disorders are more common than appreciated by most physicians. The overall incidence is 1-2% of newborns.1 Over 300 IEM are now recognised and the number is increasing.2

Most IEM are progressive and manifest after a variable symptom-free interval. Appearance of symptoms coincides with variable degree of target organ damage, which at times is permanent. Most IEM affect the brain and as a consequence majority are associated with neurological symptoms. Metabolic disorders account for 43% of the causes of mental retardation and a majority result in permanent neurological disability.3 The challenge is to diagnose these disorders, especially the treatable group, at an early stage in order to prevent or minimize end-organ damage. This is often not borne out in practice with tragic consequences.

In India, IEM rank number five as contributors to the burden of genetic diseases.4 Investigations are expensive especially in the private sector. Cost of treatment is prohibitive and many modalities are not easily available. Late diagnosis and treatment takes its toll on the quality of life. Though the health economic burden has not been estimated, it could be
considerable in terms of direct costs and indirect costs from loss of productivity. The thrust in India in the past decade was prevention of recurrence by prenatal diagnosis (PND) and termination of affected pregnancies in families having an affected child. However, recently an increasing number of individuals with IEM especially lysosomal storage disorders (LSDs) are being treated. If the quality of life were to improve in these children, the therapy has to commence as early as feasible in the presymptomatic phase. Thus early diagnosis has to be facilitated. One option available is newborn screening or by prenatal diagnosis at an even earlier stage. There is an urgent need in India to examine scope for newborn screening for IEM.

The planning of prenatal diagnostic services and newborn screening should take into consideration the common disorders prevalent in the country. No population based data is available and most information is from genetic centres in India and laboratory based results of high risk testing. Amongst the single gene disorders the common IEM were mucopolysaccharidosis (MPS), metachromatic leukodystrophy, oculocutaneous albinism, Wilson disease and aminoacid disorders. The common amino acid disorders are hyperglycinemia, homocystinuria, alkaptonuria and maple syrup urine disease (MSUD). MPS (37%), LSDs (24%), Wilson disease (14%), galactosemia (5%) and glycogen storage disease (4%) were the commonest disorders at KEM Hospital, Mumbai and AIIMS, New Delhi. The scope of this article is to familiarize the reader with facilities for prenatal diagnosis and status of newborn screening in India.

Prenatal Diagnosis

PND of a genetic disease was first reported in 1968 when Fujimoto, et al correctly identified a carrier female fetus of X linked hypoxanthine guanine phosphoribosyl transferase deficiency by biochemical analysis of cultured amniotic fluid cells. PND has now evolved as a safe and accurate option for almost all IEM. Since the past decade, prenatal diagnosis for LSDs has been fairly well established in India. Several centres (KEM Hospital in Mumbai, AIIMS and Sir Gangaram Hospital in New Delhi, Centre for DNA fingerprinting and diagnostics in Hyderabad and FRIGE in Ahmedabad) are routinely offering PND services for LSDs by estimation of enzyme activity. Reports of PND for a few other IEM like phenylketonuria and tyrosinemia type 1 have been published from Sir Gangaram Hospital, New Delhi.

PND offers prospective parents the assurance of having an unaffected child with an option for medical termination for an affected pregnancy for IEM that are lethal, incurable and resulting in chronic disability. When viewed from this context PND for treatable disorders such as Wilson disease may raise ethical issues. However, the scope of PND has been currently extended by the increasing availability of disease specific therapies such as enzyme replacement therapy for LSDs, as it can ‘prepare’ the parents for the financial and practical implications of the therapeutic options. The advantage would be commencing therapy soon after birth, which would prevent target organ injury. It is expected that the eventual long-term outcome of such presymptomatic therapy would be optimum.

For an IEM, PND should be offered when the diagnosis has been confirmed (by estimation of enzyme activity, genotyping or histopathology) in a previously affected child or the carrier status of the parents for an autosomal recessive disease has been established or a woman is known to be a carrier or is at risk of being a carrier of a X-linked recessive disease. In India the diagnosis is often not established in the previous affected child and the clinical records may not be preserved. In fact, many couples with a
previously affected child present to a genetic service only when the wife is pregnant. Under these circumstances, PND has been offered for the most likely disorder based on clinical features and available records after adequate counselling. The family should be made to understand the limitation/inaccuracy inherent with this approach. In case of enzyme estimation or histopathology as techniques for prenatal diagnosis, the defect must be expressed in fetal tissues used for sampling. For example, PND for disorders such as PKU, ornithine transcarbamylase deficiency or Von Gierke’s disease cannot be performed by enzyme estimation of chorion villus or amniotic fluid fibroblasts as the enzyme is only expressed in the liver. Genotyping will be the method of choice for such disorders. Pregnancies with non-immune fetal hydrops are at risk for IEM and are candidates for PND (Table 1). It is desirable that parents are willing for termination of an affected pregnancy without personal, social or religious taboos if options for therapy are not available or the outcome with therapy is suboptimum. However, this may not be an absolute requirement and the family’s decision has to be respected. In such cases invasive tests should be avoided if a non-invasive option is available. Pre-test counselling for risk of occurrence / recurrence of the disorder versus risk of the procedure (in case of an invasive test) is required. The couple should give informed consent for the procedure. The legal provisions under the Prenatal Diagnostic Techniques Act (PNDT) should be fulfilled. According to the amendments in the PNDT Act, fetal sex determination may be allowed under exceptional circumstances. This would include X-linked recessive disorders such as Hunter syndrome, adrenoleukodystrophy or Fabry disease. For these disorders, if PND is not possible by estimation of enzyme activity or genotyping, an option would be fetal sex determination with termination of the male fetus which has a 50% risk of being affected. Once the results of PND are available, a post-test counselling session has to be scheduled.

Modalities for prenatal diagnosis

I) Fetal imaging

A) Ultrasonography (USG): Information provided includes fetal viability, number of gestations, duration of pregnancy and fetal/placental position to plan invasive procedures. USG also provides an assessment of the fetal well being and a quantitative assessment of the fetal and the placental blood flow using Doppler. Fetal or placental structural abnormalities secondary to IEM can be detected by USG (Table 1). Limitations of USG are that it is considerably operator and interpreter oriented. It depends on the fetal position and the bone and soft tissue windows and provides a smaller field of vision as compared to MRI. Utility of USG in obese women or for the study of intracranial anatomy is limited. Fetal visualization has improved with recent use of 3-dimensional USG which allows several planes to be viewed and the images to be rotated. 3D ultrasound is currently used as an adjunct to 2D ultrasound and is particularly useful for evaluating dysmorphic features, coarse facial features, facial clefts, spinal abnormalities, hand abnormalities and determination of fetal gender in counselling pregnancies at risk for X-linked recessive disorders.

B) Fetal MRI: Currently, fetal MRI is an adjunct to USG. It is useful in the study of subtle or inconclusive lesions detected by USG and is especially advantageous in studying brain maturation and myelination. Advantages offered by MRI are that fetal visualization is not
limited by maternal obesity, oligohydramnios or fetal position and it offers superior visualization of the brain. MRI is usually performed after 20 weeks of gestation. Structural brain malformations such as corpus callosum agenesis/dysgenesis can be detected in fetuses with pyruvate dehydrogenase deficiency, non-ketotic hyperglycinemia and maternal phenylketonuria. Peroxisomal disorders and fatty acid oxidation defects can produce migration defects and holoprosencephaly may be seen in defects of cholesterol metabolism.\(^{13,14}\)

**II) Invasive modalities**

A) Histopathology of fetal tissues or placenta:

Prenatal diagnosis of mucolipidosis type II (I-cell disease) can be performed quickly and reliably by electron microscopy of chorionic villus tissue.\(^{15}\)

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>IEM to be suspected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased nuchal translucency</td>
<td>Mucopolysaccharidosis type VII</td>
</tr>
<tr>
<td>Fetal hydrops</td>
<td>Mucopolysaccharidosis type VII and IV, Mucolipidosis type I and II, GM₃ gangliosidosis, Gaucher disease, Niemann-Pick disease type C and A, Infantile sialic acid storage disease, Galactosialidosis, Farber disease, Multiple sulphatase deficiency, Wolman disease</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>Gaucher disease type 2, Niemann-Pick disease</td>
</tr>
<tr>
<td>Fractures</td>
<td>I-cell disease</td>
</tr>
<tr>
<td>Placenta: Pale and bulky</td>
<td>Lysosomal storage disorders</td>
</tr>
<tr>
<td>Polydactyly/syndactyly/cleft lip</td>
<td>Smith Lemli Opitz</td>
</tr>
<tr>
<td>Reduced or absent fetal movements</td>
<td>Nonketotic hyperglycinemia</td>
</tr>
</tbody>
</table>

B) Amniocentesis

- Cell free amniotic fluid: Identification of storage material or substrates (glycosaminoglycans, oligosaccharides, sialic acid, organic acid metabolites) in supernatant

  - Amniotic fluid cells
    - Uncultured amniocytes
    - Cultured amniocytes

C) Chorion villus sampling (CVS)

  Uncultured cells

  Cultured cells

CVS and cultured amniotic fluid cells are used either for enzyme estimation or to obtain fetal DNA for genotyping.
If a mutation has been identified in the proband, the best option would be fetal genotyping. However, in India there are rare instances where the proband’s genotyping is available. In this case fetal DNA diagnosis is still possible by detecting a common mutation in a given population, e.g. L444P in neuronopathic Gaucher disease. It must be realized that for a given IEM there are ethnic variations in disease causing mutations. Apart from some common conditions like Gaucher disease and glycogen storage disease type I, mutations in Indian patients have not been studied. Moreover many disorders demonstrate private mutations. If a laboratory tests only common mutations or mutations of an ethnically diverse population (e.g. N370S Gaucher mutation seen in Ashkenazi Jews, but rare in non-Jews), its absence may not rule out an affected fetus. In this case DNA sequencing is required to study the entire coding region of the gene of interest. Moreover some changes may be mere polymorphisms and therefore non-pathogenic. In this case presence of an alteration may not indicate disease. Knowledge of local disease genotype and physician’s knowledge of the limitations of a given diagnostic test is crucial to interpret results and counsel families accordingly.

D) Fetal blood sampling: By 18 weeks of gestation fetal blood may be obtained by cordocentesis. An example is diagnosis of arginemia by the estimation of fetal erythrocyte arginase levels.5

E) Fetal tissues: Fetal tissues may be biopsied during fetoscopy for the diagnosis of certain IEM like oculocutaneous albinism and Von Gierke disease (liver).16,17

The pediatricians’ role would be to ensure confirmed diagnosis in the index case. Being responsible for the long-term medical care of these children, the families develop a rapport and a relationship of trust with the primary care pediatrician. This places the pediatrician in an ideal position for counselling and referring such couples to a prenatal screening or diagnostic service in time.

NEW BORN SCREENING

Newborn screening (NBS) was conceived as a public health program in which every newborn is tested for early identification of a selective panel of disorders based on the premise that early intervention of affected newborns before symptomatic disease will lead to a significant reduction in mortality and associated disabilities.

It is an integrated system of education (health professionals, parents, general public and politicians), screening (sample collection, transport, laboratory testing and reporting), early follow-up (notification of abnormal results, tracking and confirmation), diagnosis, management (monitoring, genetic counselling and long term follow-up) and evaluation (quality assurance and outcome).27

Since Robert Guthrie first introduced NBS to detect PKU in 1961 followed by screening for congenital hypothyroidism (CH) in the mid 70s, the benefits to the individual, family and society have been proven beyond doubt.28,29 Encouraged by the success for these two disorders many countries adapted the program including Asian countries like China, Korea, Thailand, Philippines and Singapore.27 Many disorders like galactosemia, MSUD, homocystinuria, biotinidase deficiency, sickle cell disease and tyrosinemia have been added to the screening panel depending on the policy of an individual country or state.29 In contrast to conventional NBS, expanded screening detects a large number of disorders in a single analytical run. This was possible by adaptation of tandem mass spectrometry (MS/MS) for newborn screening
Table 2. IEM detected by newborn screening

<table>
<thead>
<tr>
<th>Condition</th>
<th>Incidence</th>
<th>Type of test</th>
<th>Analytes tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylketonuria, hyperphenylalaninemia</td>
<td>1:12000 to 1:15000</td>
<td>BIA, C, F, MS/MS</td>
<td>Phenylalanine, tyrosine</td>
</tr>
<tr>
<td>Maple syrup urine disease</td>
<td>1:250000</td>
<td>BIA, F, MS/MS</td>
<td>Leucine or valine &amp; leucine/ isoleucine</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>1:250000</td>
<td>BIA, C, MS/MS</td>
<td>Homocysteine or methionine</td>
</tr>
<tr>
<td>Tyrosinemia (type 1)</td>
<td>1:150000</td>
<td>BIA, C, MS/MS</td>
<td>Tyrosine, methionine (type 1)</td>
</tr>
<tr>
<td>Galactosemia (classical)</td>
<td>1:60000</td>
<td>BIA, F</td>
<td>Galactose, galactose-1-phosphate</td>
</tr>
<tr>
<td>Biotinidase deficiency</td>
<td>1:250000</td>
<td>F</td>
<td>Biotinidase enzyme activity</td>
</tr>
<tr>
<td>Organic acidemias (Glutaric acidemia type I and II, isovaleric acidemia, propionic academia, methylmalonic academia, disorders of ketogenesis and ketolysis)</td>
<td></td>
<td>MS/MS</td>
<td>Respective target acylcarnitine concentration</td>
</tr>
<tr>
<td>Urea cycle defects</td>
<td></td>
<td>MS/MS</td>
<td>Respective target amino acid concentrations</td>
</tr>
<tr>
<td>Medium chain acyl CoA dehydrogenase deficiency</td>
<td>1:14600</td>
<td>MS/MS</td>
<td>C6, C8, C10, C10:1, C8/C10 ratio</td>
</tr>
<tr>
<td>Lysosomal storage disorders (Pompe, Gaucher, Fabry, MPS I, Krabbe, Niemann-Pick type A &amp; B)</td>
<td>1:7000 (cumulative)</td>
<td>MS/MS</td>
<td>Respective enzyme activity</td>
</tr>
</tbody>
</table>

BIA= bacterial inhibition assay, C= chromatography, F=fluorometric,

In the 90s, IEM detected by newborn screening and testing modality are given in Table 2. Prenatal diagnostic modalities for disorders of carbohydrate metabolism, lysosomal disorders, disorders of aminoacid / organic acid and other disorders are given in Annexures 1, 2, 3 and 4 respectively.

In India newborn screening for IEM like organic acidemias, urea cycle defects and amnioacidopathies is presently practised in at risk families with previous undiagnosed neonatal deaths, sudden infant deaths or acute metabolic encephalopathy. Despite the evidence world-over, the concept of universal NBS has not
been accorded due priority, either by the government or the neonatal and pediatric professional bodies. In an editorial in Indian Pediatrics Morris has commented that “having the technology to undertake neonatal screening for a range of metabolic disorders does not mean that this is the right thing to do. To answer this question, one needs local information about incidence, natural history and effectiveness of treatment”. He concluded that neonatal screening for these conditions would be less cost-effective in India than in the UK. In light of these critical observations, there is a need for pilot programs to answer these doubts.

In the earliest study in Karnataka, 112369 newborns were screened for aminoacidurias. Disorders identified most frequently were tyrosinemia (1 in 6243), MSUD (1 in 10215), glycineuria (1 in 26053) and PKU (1 in 28728). From the year 2000 onwards, an expanded program of newborn screening was performed in Andhra Pradesh. A total of 18,300 newborns were screened for aminoacidopathies, CH, congenital adrenal hyperplasia (CAH), galactosemia, glucose-6-phosphate dehydrogenase deficiency (G6PD), biotinidase deficiency and cystic fibrosis. The study revealed an astounding high incidence of CH (1 in 1700) and CAH (1 in 2600) as compared to world-wide data. A small project to screen for CH in Kochi, Kerala has revealed an even higher incidence of 2.1 per 1000. The comparative frequency in other parts of the world for CH is 1 in 3600 in the USA, 1 in 7000 in Scandinavia, 1 in 3000 in Europe and 1 in 5700 in Japan and the incidence for CAH is 1 in 288 in Yupik Eskimos, 1 in 7000 in Philippines, 1 in 12000 in USA, 1 in 5500 to 1 in 10000 in Italy, and 1 in 15000 in Japan. Recently, the State of Goa has taken the lead in initiating expanded mass screening for newborns as a public-private partnership with a Bangalore based laboratory service. The program “Heel to Heal” launched on 14th June 2008 will screen for a panel of 45 disorders. Three months following commencement of the program, the incidence rate in Goa was 1 in 400 and the disorders detected were urea cycle defects, methylmalonic /propionic acidemia, VLCADD, G6PD and CH.

Thus there is no reason to assume that IEM are less frequent in India than elsewhere in the world. With this overwhelming data, the Indian council of Medical Research has taken the initiative of constituting a Multicentric Task Force Group for inborn metabolic disorders. Newborn screening was identified as a priority. A multicentric pilot project for NBS was commenced in Chennai, Hyderabad, Kolkata, Mumbai and New Delhi with the aim of screening 100000 newborns for CH and CAH to study among others the incidence and feasibility of implementing newborn screening on a nationwide scale in India.

**Newborn screening technology for IEM**

The earliest first generation technology was bacterial inhibition assay (BIA) pioneered by Dr. Robert Guthrie. He also developed the dried blood spot (DBS) technique of collecting blood on a special filter paper from a heel prick. This process was manual and took up to 24 hours. With introduction of automation, second generation fluorometric or colorimetric assays were developed (Table 2). Alternative techniques are chromatographic (thin layer chromatography for amino acids or organic acids or high pressure liquid chromatography (HPLC). Introduction of MS/MS for newborn screening was the third generation technology for simultaneous assays for aminoacidurias, organic acidemias and fatty acid oxidation defects. Its application for NBS analyzes multiple analytes in less than 3 minutes and has a sensitivity of 100% and specificity of 99% for.
some disorders. However, not all the metabolites detected may be analyzed or reported. Each state or country selects target metabolites for reporting. In Switzerland and UK, only PKU and MCAD are targeted, whereas Germany and Austria report 10 and 20 respectively and in the USA 29 disorders are primary targets whereas 25 additional disorders are secondary targets. Some disorders such as SCAD, 3-methyl-CoA-carboxylase deficiency detected by MS/MS are clinically inconsequential and may not be reported. The technology has recently been adapted to screen lysosomal storage disorders. Simultaneous detection of a panel of six disorders (Gaucher, Fabry, Pompe, MPS I, Krabbe and Niemann-Pick disease type A & B) is now well established from a dry blood spot (DBS) sample (multiplex testing). Assays are now being developed for Tay Sachs disease, MPS II, MPS VI and metachromatic leukodystrophy. A relative disadvantage of MS/MS is that congenital hypothyroidism and galactosemia cannot be tested.

It must be clear that an elevated analyte concentration from the original specimen requires tracking the child to obtain a second DBS. If the analyte is abnormal in the second assay, the baby has to be referred for confirmatory tests and therapy. Thus it should be understood that merely detecting an abnormal analyte from a DBS is not diagnostic and must be followed by confirmatory tests. Confirmatory tests would include aminoacid measurement by a quantitative technique like HPLC or estimation of enzyme activity or genotyping.

**Selection of disorders to screen**

In 1968, Wilson and Jungner described guidelines for selecting disorders for screening. The disorders selected for NBS should be severe diseases relatively frequent in a given population, treatable or controllable, those for which a test exists and a suitable specimen can be obtained easily. It is necessary that manifestations appear after a variable symptom-free interval. Phenylketonuria was the prototype. It is significantly prevalent in North America and Europe with an incidence of 1 in 12000 live births. Early manifestations such as seizures, eczema and depigmented hair and skin occur in the first few months of life and untreated disease leads to severe mental retardation, hyperactivity, aggressive behaviour and autism. Blood phenylalanine levels in an affected infant are abnormally high by 72 hours of life. Simple methods can detect blood phenylalanine levels from a DBS (Table 6). Effective therapy in the form of medical foods complemented by a phenylalanine restricted diet begun by 3 weeks of life prevents mental retardation and neurological abnormalities. While PKU and CH are models fulfilling these requirements, the benefits of screening for most other disorders by strictly adhering to these criteria is less evident. Many newborns with MSUD, salt wasting CAH, classical galactosemia and organic acidemias like propionic acidemia may develop life-threatening symptoms even before results of newborn screening are available. In the latter two disorders though early identification by screening prevents death and complications, it does not prevent long-term sequels. Well-treated children with galactosemia develop speech and behavioural abnormalities, visual perceptual learning abnormalities and ovarian failure is not prevented. The experience with the severe variants of propionic acidemia is similar - neurocognitive disability is not completely prevented and acute relapses occur, though less frequently. Pyridoxine-responsive homocystinuria may not have elevation of methionine, the target analyte in the first days of life, and may therefore be missed. Thus the benefits of NBS for these disorders is less evident. Nevertheless, strict adherence to these criteria cannot hold
enough ground in today’s era. High prevalence of a condition as a screening criterion is irrelevant if the diagnosis comes ‘free’ with multiplex testing for other disorders, as is the case with tandem mass spectroscopy that simultaneously detects up to 45 disorders from a single blood spot. Moreover pressure from the public or support groups may be the driving force in selection of disorders for screening. It has been revealed that parents may want to know if their newborn is affected irrespective of prospects for treatment. Their future reproductive planning, for e.g. prenatal diagnosis or undergoing tubal ligation, may be based on the availability of screening for an untreatable disorder or they may simply want to avoid the psychological trauma of a wrong early diagnosis or may adjust emotionally and financially to the presence and treatment of a disease in their infant at an early stage.

**Challenges for newborn screening in India**

**Prospects for countrywide screening for IEM**

From the Indian perspective, there is no debate over the urgency for countrywide screening for CH from the high incidence even in areas not traditionally known to have endemic iodine deficiency. It is one of the first disorders to be screened in countries beginning NBS programs. Similarly, incidence of G6PD deficiency is 2% to 7.8% and accounts for 32% of neonates with hyperbilirubunemia. In common with several South Asian countries, NBS for G6PD deficiency would be an easy choice. The same would be true for beta thalassemia. A decision to implement countrywide NBS for IEM, even those with a gratifying success like PKU would be less straightforward. This would stem from the lack of easy access to therapy in the form of medical foods. Difficulty in procuring medical foods due to lack of appropriate legislation, government subsidy or health insurance reforms has prompted criticism for screening for some IEM in the USA. The same argument would discourage NBS for lysosomal storage disorders till such a time that therapy is available and affordable in the country. Until the natural history and benefits of NBS for organic acidemias like propionic acidemia or MCAD is clearly evident from countries screening these disorders, adopting these as targets for screening in India may have to be deferred.

**Prospects for screening for IEM in individual States**

The wide disparity in demographics and health performance indicators within India makes a uniform countrywide policy for NBS impractical. Difficulties would arise from a large geographic area, wide variations in terrain with inaccessible areas like mountainous terrain and tribal belts, inter-state socioeconomic disparity, rural to urban migrations, illiteracy and religious and cultural taboos. Therefore it would seem wiser for NBS to be prioritized in each state according to their demographics; health expenditure projections; infrastructure in terms of technology, clinical and laboratory expertise, manpower and organizational capability for delivery of the program.

From a demographic perspective, there are three regions based on the infant mortality and literacy rates. The first is Goa and Kerala, the only two states in the country with an IMR of <20 and literacy rate of 83% and > 90% respectively (Census of India 2001, http://cyberjournalist.org.in/census/cenlit0.html). Since June 2008, Goa has initiated universal NBS as a public-private partnership for 45 metabolic disorders in addition to CH, CAH, G6PD deficiency and cystic fibrosis. Kerala has yet to initiate NBS on state-wide basis. In these states further reduction in IMR to < 5 like Singapore and Japan would be feasible only with measures
to eliminate mortality due to genetic disorders like IEM and malformations. Therefore NBS must be a priority in Kerala. Maharashtra (IMR 35, literacy rate 77%), Tamil Nadu (IMR 37, literacy rate 73%) and New Delhi (IMR 37, literacy rate 81%) fall in the second region. In these states, NBS is an emerging priority. Implementing NBS in the next few years in these states will also ensure that the IMR of < 30 per 1000 is quickly achieved. The rest of the country, with an IMR > 40 comprises the third section where infectious disease, perinatal asphyxia and neonatal sepsis still take a heavy toll. NBS will not be a priority in such states.

Hurdles to be overcome in India

Factors identified for successful implementation of a screening program are government prioritization, government financing, public education and acceptance, co-operation and involvement of health practitioners, government participation in institutionalizing a screening system and integration into the existing national health care system. Delivery of health care in India is through the public and private sectors. Under the public health programs health care delivery differs in the rural and urban areas. Rural population is served by a vast network of primary health centres and sub-centres in India. With training, health resources and manpower at the primary health care level can be galvanized for implementation of NBS. This approach was shown to be practical and feasible by Kochupillai et al in a rural setting in Uttar Pradesh. On the contrary, implementation in the city using the existing infrastructure of a Pediatric referral hospital in Mumbai was fraught with organizational difficulties: 28% missed collections, a dismal 30% response to recall, delayed reporting/ follow-up examination, migratory slum population and illiteracy leading to difficulty in recording addresses. The number of institutional deliveries in India is presently 40.7%. (NFHS 3) The program will have to make provisions for covering 60% of the newborns delivered at home into the NBS program. Currently, one third of all deliveries are attended by traditional birth attendants (TBA) and 48.3% of deliveries are assisted by health professionals (doctor / nurse / LHV / ANM / other health personnel). These birth attendants will have to be educated and trained for motivating families and for DBS collection and ensuring that it reaches the laboratory. For program success, the tracking and follow-up of positive screens is crucial. Efficient tracking of screen-positive babies would have to be implemented on the lines of AFP surveillance. Developing infrastructure in terms of trained manpower (laboratory as well as clinical expertise), investment in technology for confirmation and monitoring and easy availability of therapeutic options is the logical accompaniment for success of program. On ethical grounds, it would be unreasonable to invest in screening and diagnostic facilities without ensuring easy availability of therapy in the form medical foods (Aminoacidopathies, organic acidemias, urea cycle defects), enzyme replacement therapy (lysosomal storage disorders) or organ transplantation (bone marrow, liver, kidney, stem cell). Opponents of newborn screening would cite these deficiencies and diverting health expenditure in implementation of the program in India. In countries of Asia-Pacific, the cost of screening varies from no fees (Palau) to USD 32 (Singapore). It is paid by the Government in Australia, Bangladesh, Hong Kong, Japan, New Zealand, Sri Lanka, Thailand and South Korea while the family bears the cost in China, Taiwan, Indonesia, Philippines and Singapore. In India, the cost is borne by the family or in the case of Goa directly or indirectly by the Government including cost for confirmatory tests and medical foods for those screening positive and confirmed to have an IEM. Confirmatory tests for a few selected screen
positive disorders are obtained through the testing laboratory (personal communication). An option would be mandating payment for the cost of screening and therapy through insurance reforms by legislation. The program will also have to address the question – whether NBS should be mandated like in China and Philippines or voluntary? Some states in the USA mandate NBS by legislation. If parents refuse testing citing religious or other reasons, an “informed dissent” document is required to be signed.35

The need of the day would be to set up an Expert Group on newborn screening under the auspices of professional bodies like National Neonatology Forum and Indian Academy of Pediatrics. This group would have the necessary authority to represent the cause of NBS to the government. It would also play a proactive role in propagating the cause of newborn screening in the country for training, awareness and educational campaigns for pediatricians and other relevant health care professionals on the lines of breast feeding training, IMNCI and HIV programs.

Points to Remember

• **Prenatal diagnosis for IEM should be offered to families for prevention of recurrence when the diagnosis is confirmed in an index case.**

• **Abnormalities such as fetal hydrops or visceromegaly detected by ultrasonography should prompt investigations for an IEM under appropriate circumstances.**

• **The appropriate option for prenatal diagnosis is chorion villus sampling or amniocentesis for estimation of enzyme activity or genotyping if the mutations have been tested in the index case. In other cases options such as substrate or metabolite profiling in the amniotic fluid supernatant may be appropriate.**

• **Newborn screening for IEM permits early diagnosis and treatment resulting in prevention of disabilities, especially neurodisability.**

• **Implementing universal newborn screening would lead to substantial gains in decreasing the infant mortality rates.**

Acknowledgement

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Annexure 1. Prenatal diagnosis for disorders of carbohydrate metabolism

<table>
<thead>
<tr>
<th>Disease</th>
<th>Inheritance</th>
<th>Sample</th>
<th>Diagnostic assay</th>
<th>Special remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactosemia</td>
<td>AR</td>
<td>AFC</td>
<td>(E) Galactokinase</td>
<td></td>
</tr>
<tr>
<td>Galactosemia</td>
<td>AR</td>
<td>CVS, AFC, AF</td>
<td>(E) Galactose 1-phosphate uridyl transferase</td>
<td>Raised galactitol using GC/MS in 1st/2nd trimester</td>
</tr>
<tr>
<td>Glycogenosis Ia</td>
<td>AR</td>
<td>AFC, CVS</td>
<td>M (E) Glucose 6-phosphatase</td>
<td>Mutation analysis now obviates the need for a invasive fetal liver biopsy</td>
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<td>Glycogenosis III</td>
<td>AR</td>
<td>CVS,AFC</td>
<td>(E) Amylo 1,6 glucosidase M</td>
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<td>Glycogenosis IV</td>
<td>AR</td>
<td>CVC, AFC</td>
<td>(E) Brancher enzyme M</td>
<td>USG- hydrops fetalis</td>
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<td>Chromosome locus</td>
<td>Gene</td>
<td>Prenatal diagnosis</td>
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<td>-----------------------------</td>
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<tr>
<td>GM1 Gangliosidosis</td>
<td>*ß-Galactosidase</td>
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<td>GLB1</td>
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<td>15q</td>
<td>HEX A</td>
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<td>Sandhoff Disease</td>
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<td>5q13</td>
<td>HEX B</td>
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<td>GM2 Gangliosidosis AB variant</td>
<td>GM2 activator protein</td>
<td>5q</td>
<td>GM2A</td>
<td></td>
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<td>Niemann Pick Types A and B</td>
<td>*Sphingomyelinase</td>
<td>11p15</td>
<td>SMPD1, NPD</td>
<td>E, M</td>
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<td>Cholesterol esterification defect</td>
<td></td>
<td>NPC1, NPC2/HE1</td>
<td>Cholesterol esterification in CC, M, H</td>
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<tr>
<td>Gaucher Disease</td>
<td>*Glucocerebrosidase</td>
<td>1q21-q31</td>
<td>GBA</td>
<td>E, M</td>
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<td>Gaucher disease</td>
<td>Saposin C</td>
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<tr>
<td>Metachromatic Leukodystrophy</td>
<td>*Arylsulfatase A</td>
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<td>E, M</td>
</tr>
<tr>
<td>Metachromatic Leukodystrophy</td>
<td>Saposin B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Krabbe Disease</td>
<td>*Galactocerebrosidase</td>
<td>14q31</td>
<td>GALC</td>
<td>E, M</td>
</tr>
<tr>
<td>Fabry disease#</td>
<td>A-galactosidase</td>
<td>Xq22</td>
<td>a-Gal A</td>
<td>E, M</td>
</tr>
<tr>
<td>Farber Disease</td>
<td>*Ceramidase</td>
<td>8P</td>
<td>ASAH</td>
<td>E, M</td>
</tr>
<tr>
<td>Wolman Disease</td>
<td>Acid Lipase</td>
<td>10q24</td>
<td>LIPA</td>
<td>E*</td>
</tr>
<tr>
<td>MPS I</td>
<td>*a-L-Iduronidase</td>
<td>4p16</td>
<td>IDUA, IDA</td>
<td>AFS, E, M</td>
</tr>
<tr>
<td>MPS II#</td>
<td>*Iduronate Sulfatase</td>
<td>Xq28</td>
<td>IDS</td>
<td>AFS, E, M</td>
</tr>
<tr>
<td>MPS IIIA</td>
<td>Heparan N-Sulfatase</td>
<td>17q25</td>
<td>SGS, MPS3A, SFMD</td>
<td>AFS, E, M, radioactive sulphamidase assay</td>
</tr>
<tr>
<td>MPS IIIB</td>
<td>N-acetylglucosaminidase</td>
<td>17q21</td>
<td>NAGLU</td>
<td>AFS, E, M</td>
</tr>
<tr>
<td>MPS IIIC</td>
<td>Acetyl-CoA-Glucosaminide</td>
<td>8p11-q13</td>
<td>MPS3C</td>
<td>AFS, E, M</td>
</tr>
<tr>
<td>MPSIIID</td>
<td>N-acetylglucosamine-6-sulfatase</td>
<td>12q14</td>
<td>GNS, G6S</td>
<td></td>
</tr>
<tr>
<td>MPS IVA</td>
<td>Galactosamine-6-Sulfatase</td>
<td>16q24</td>
<td>GALNS</td>
<td>AFS, E, M</td>
</tr>
<tr>
<td>MPS IVB</td>
<td>*ß -Galactosidase</td>
<td>3p21</td>
<td>GLB1</td>
<td>AFS, E, M</td>
</tr>
<tr>
<td>MPS VI</td>
<td>Arylsulfatase B</td>
<td>5q13</td>
<td>ARSB, MPS6</td>
<td>AFS, E, M</td>
</tr>
</tbody>
</table>

Contd...
<table>
<thead>
<tr>
<th>Disease</th>
<th>Functional defect/deficiency</th>
<th>Chromosome locus</th>
<th>Gene</th>
<th>Prenatal diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPS VII</td>
<td>*β -Glucuronidase</td>
<td>7q21</td>
<td>GUSB, MPS7</td>
<td>E, M</td>
</tr>
<tr>
<td>MPS IX</td>
<td>Hyaluronidase</td>
<td>3p21</td>
<td>HYAL1</td>
<td>-</td>
</tr>
<tr>
<td>Mannosidosis</td>
<td>a-Mannosidase</td>
<td>19cen-Q12</td>
<td>MAN2B1, MANB</td>
<td>E, M</td>
</tr>
<tr>
<td>Fucosidosis</td>
<td>a-L-Fucosidase</td>
<td>1p34</td>
<td>FUCA1</td>
<td>E, M</td>
</tr>
<tr>
<td>Aspartylglycosaminuria</td>
<td>N-Aspartyl-b-Glucosaminidase</td>
<td>4q32</td>
<td>AGA</td>
<td>E</td>
</tr>
<tr>
<td>Sialidosis (Mucolipidosis I)</td>
<td>Neuraminidase</td>
<td>6p21</td>
<td>NEU1, SIAL1</td>
<td>CC, M</td>
</tr>
<tr>
<td>Mucolipidosis II (I-Cell Disease)</td>
<td>N-Acetylglucosamine-1-Phosphotransferase</td>
<td>4q</td>
<td>GNPTA</td>
<td>AFS, CC, M, H</td>
</tr>
<tr>
<td>Mucolipidosis IIIA (Pseudo-Hurler Polydystrophy)</td>
<td>N-Acetylglucosamine-1-Phosphotransferase</td>
<td>4q</td>
<td>GNPTAB</td>
<td>AFS, CC, M</td>
</tr>
<tr>
<td>Mucolipidosis IIIC</td>
<td>N-Acetylglucosamine-1-Phosphotransferase</td>
<td>16p</td>
<td>GNPTAG</td>
<td></td>
</tr>
<tr>
<td>Mucolipidosis IV</td>
<td>Mucolipin-1</td>
<td>19p13</td>
<td>MCOLN1</td>
<td>AFC</td>
</tr>
<tr>
<td>Galactosialidosis</td>
<td>Lysosomal protective protein (cathepsin A)</td>
<td>20q13</td>
<td>PPGB, GSL, NGBE, CTSA, GLB2</td>
<td>E</td>
</tr>
<tr>
<td>Schindler Disease</td>
<td>a-N-Acetyl-Galactosaminidase</td>
<td>22q11</td>
<td>NAGA</td>
<td>E</td>
</tr>
<tr>
<td>Pompe Disease</td>
<td>Acid a-1, 4-Glucosidase</td>
<td>17q25</td>
<td>GAA</td>
<td>E, M</td>
</tr>
<tr>
<td>Infantile sialic acid storage disease</td>
<td>Sialic acid transporter</td>
<td>6q</td>
<td>SLC17A5, SIASD, SLD</td>
<td>AFS, M</td>
</tr>
<tr>
<td>Salla Disease</td>
<td>Sialic acid transporter</td>
<td>6q</td>
<td>SLC17A5, SIASD, SLD</td>
<td>AFS, M</td>
</tr>
<tr>
<td>Cystinosis Danon disease</td>
<td>Cystine Transporter LAMP-2</td>
<td>17p13</td>
<td>CTNS, LAMP2, LAMB</td>
<td>CC</td>
</tr>
<tr>
<td>NCL infantile type</td>
<td>Palmitoyl protein thioesterase</td>
<td>1p32</td>
<td>PPT1, CLN 1</td>
<td>M</td>
</tr>
</tbody>
</table>

Contd...
Disease Functional Chr omosome Gene Prenatal
defect/deficiency locus diagnosis

NCL late infantile type Tripeptidyl peptidase 11p15 CLN 2 M
Juvenile NCL Adult NCL Membrane protein Palmitoyl protein 16p12 CLN 3 M
(Kufs disease) NCL Finnish late infantile type thioesterase Membrane protein 1p32 13q CLN 4 CLN 5 - M
NCL early juvenile - 15q CLN 6 -
NCL - CLN 7 -
NCL Membrane protein CLN 8 -
Multiple sulphatase deficiency Sulphatase modifier protein (Arylsuphatase A, B, steroid sulphatase enzyme activities)

# Fetal sexing is essential to identify unaffected female heterozygotes in whom low enzyme activity may overlap in the affected range.

AF = Amniotic Fluid, AFC = Cultured amniotic fluid cells, AFS = Amniotic fluid supernatant, CVS = Chorion villus sampling, CC = Cultured chorionic villi, M = Mutation, AR = Autosomal recessive E = Enzyme analysis

* Prenatal diagnosis for these disorders is available at several genetic centers in India by estimation of enzyme activity from chorionic villus tissue or cultured amniotic fluid fibroblasts.

Annexure 3. Prenatal diagnosis for disorders of amino acid and organic acid metabolism

<table>
<thead>
<tr>
<th>Disease</th>
<th>Inheritance</th>
<th>Sample</th>
<th>Diagnostic assay</th>
<th>Special remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylketonuria 1</td>
<td>AR</td>
<td>AFC, CVS</td>
<td>M</td>
<td>3/4th of the affected cases are likely to be genetic compounds of PKU alleles20</td>
</tr>
<tr>
<td>Phenylketonuria 2</td>
<td>AR</td>
<td>AFC</td>
<td>(E) Dihydropteridine reductase deficiency</td>
<td></td>
</tr>
<tr>
<td>Tyrosinemia type I</td>
<td>AR</td>
<td>CVS</td>
<td>(E) Fumarylacet oacetase M</td>
<td>AFS: Succinylacetone</td>
</tr>
<tr>
<td>Albinism, oculocutaneous</td>
<td>AR</td>
<td>AFS, CVS, Fetal Skin biopsy</td>
<td>M, Electron microscopy</td>
<td>Fetal skin biopsy (20 weeks of gestation)16 Hair bulb analysis for melanosome development has given way to a more accurate molecular analysis21</td>
</tr>
<tr>
<td>Carbamoyl phosphate synthetase deficiency</td>
<td>AR</td>
<td>AFS, CVS, Fetal liver biopsy</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>Disease</td>
<td>Inheritance</td>
<td>Sample</td>
<td>Diagnostic assay</td>
<td>Special remarks</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>-------------</td>
<td>----------------------------</td>
<td>----------------------</td>
<td>------------------------------------------------------</td>
</tr>
<tr>
<td>Ornithine carbamoyl transferase deficiency</td>
<td>AR</td>
<td>AFS, CVS, Fetal liver biopsy</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>Arginosuccinic aciduria</td>
<td>AR</td>
<td>CVC, AFC, AFS</td>
<td>(E) Arginosuccinate lyase, M</td>
<td></td>
</tr>
<tr>
<td>Citrullinemia</td>
<td>AR</td>
<td>CVC, AFC, AFS</td>
<td>M</td>
<td>Radiolabelled (C(_{14})) citrulline incorporation(^{22})</td>
</tr>
<tr>
<td>Argininemia</td>
<td>AR</td>
<td>Fetal blood sample</td>
<td>(E) Arginase</td>
<td></td>
</tr>
<tr>
<td>Non ketogenic hyperglycinaemia</td>
<td>AR</td>
<td>AFS</td>
<td>Increased glycine/serine ratio</td>
<td>USG- abnormal fetal movements (reduced or absent)</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>AR</td>
<td>AFC, CVC</td>
<td>(E) Cystathionine beta synthetase</td>
<td>Uncultured cells yield poor results(^{5})</td>
</tr>
<tr>
<td>Maple syrup urine disease</td>
<td>AR</td>
<td>CVS, AFC</td>
<td>M</td>
<td>Release of CO(_{2}) from radio-labelled leucine. Uncultured cells yield poor results(^{23})</td>
</tr>
<tr>
<td>Organic acidemias (propionic acidemia, methylmalonic acidemia, glutaric acidemia type 1, ketothiolase deficiency, and isovaleric acidemia)</td>
<td>AR (majority)</td>
<td>AFS, CVS</td>
<td>E: AFC/ CVS M: AFC/ CVS Analytes: AFS</td>
<td>Electrospray ionization TMS analysis of acylcarnitines in the AFS(^{24})</td>
</tr>
<tr>
<td>Fatty acid oxidation defects</td>
<td>AR</td>
<td>CVC, AFC</td>
<td>(E)</td>
<td>Amniocyte incubation with isotope labelled precursors with acylcarnitine analysis of intermediate metabolites by TMS(^{24})</td>
</tr>
</tbody>
</table>

**Annexure 4. Prenatal diagnosis for other metabolic disorders\(^{5,26}\)**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Inheritance</th>
<th>Sample</th>
<th>Diagnostic assay</th>
<th>Special remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zellweger</td>
<td>AR</td>
<td>CVS, CVC, AFC</td>
<td>(E) Perioxosome biogenesis M</td>
<td>USG: Increased nuchal translucency, fetal hypokinesia, renal hyperechogenicity, dilated ventricles(^{25})</td>
</tr>
<tr>
<td>X Linked adrenoleukodystrophy#</td>
<td>XR</td>
<td>CVC</td>
<td>(E) Very long chain fatty acyl CoA synthetase M</td>
<td></td>
</tr>
<tr>
<td>Lesch-Nyhan</td>
<td>AR</td>
<td>CVS, AFC</td>
<td>(E) HGPRT M</td>
<td></td>
</tr>
</tbody>
</table>


# Fetal sexing is essential to identify unaffected female heterozygotes in whom low enzyme activity may overlap in the affected range
Inborn errors of metabolism (IEM) individually are rare but collectively are common. In IEM single gene defects are responsible for the abnormalities in the synthesis or catabolism of proteins, carbohydrates or fats by way of defective enzymes or transport proteins, resulting in a block of metabolic pathway. The male to female ratio is 1:1 for X-linked dominant if transmission is from mother to child.\(^1\)

For evaluating an IEM the following five important aspects should follow:

1. **History/ Family History:** We should note whether the neonate was born to consanguineous parents or not and also check history of previous siblings like fetal deaths or miscarriages or genetically affected siblings and also note the pedigree for two generations.

2. **Physical examination:** like dermatitis, alopecia, facial dysmorphism, cataract, etc.

3. **Initial screening tests:** includes complete blood count, electrolytes level, glucose, ammonia, lactate, lactate/ pyruvate ratio, reducing substances, organic acids, amino acids, ketones, mucopolysaccharides (MPS), uric acid, liver function tests (LFT), renal function tests (RFT) and Porphyrins.

4. **Advanced screening tests:** The test is performed on basis of clinical context which includes long chain fatty acids, MPS separation and specification, quantitation of amino acids, organic acids, carbohydrate and other metabolites.

5. **Definitive diagnostic tests:** To confirm the disorder, specific enzyme assays in leucocytes, plasma/serum or red cells, immunoassays and DNA probe analysis are required.

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** Minakshi Koch
** Sabyasachi Ghosh
*** Shobha G
***Suresh Kumar V

**Abstract:** Inherited metabolic disorders are a heterogeneous group of genetic conditions mostly occurring in childhood. They are individually rare but collectively numerous, causing substantial morbidity and mortality. Screening for inherited metabolic disorders is therefore very important. The importance of screening for inborn errors of metabolism (IEM) introduces several decision points about specimen collection, processing, and storage for the investigator. The method of sampling is of greatest importance for precise results and hence for earlier and accurate diagnoses.

**Keywords:** Inborn errors of metabolism, Specimen collection, Processing, Interpretation.
Individual IEM are rare disorders, most having an incidence of less than 1 per 100,000 births. However, when considered collectively, the incidence may approach 1 in 800 to 2500 births.

Age for presentation of clinical symptoms varies for individual IEM and variant forms within the IEM. The timing of presentation depends on significant accumulation of toxic metabolites or on the deficiency of substrate. The disorders of carbohydrate or protein metabolism and disorders of energy production tend to present in the neonatal period or early infancy and tend to be unrelenting and rapidly progressive. Less severe variants of these diseases usually present later in infancy or childhood and tend to be episodic. Fatty acid oxidation, glycogen storage and lysosomal storage disorders tend to present insidiously in infancy or childhood. Disorders manifested by subtle neurological or psychiatric features often go undiagnosed until adulthood.  

Screening is a basic tool for clinically suspected cases of inborn metabolic diseases by a simple economical and effective technique like paper chromatography, thin layer chromatography (TLC) and some biochemical tests. However, it is not reasonable to make firm decision on the basis of screening test. High performance liquid chromatography (HPLC), tandem mass spectrometry (TMS), gas chromatography-mass spectrometry (GC-MS) are the advanced techniques for confirming metabolic disorders. The acyl carnitines in blood reflect the primary accumulating mitochondrial acyl-CoA metabolites in disorders of fatty acid and amino acid catabolism. Thus an acyl carnitine “profile” will recognize almost all of the defects in these pathways using the advanced technique like TMS. Advanced chemical diagnosis using GC-MS has also become an important part of the routine diagnostic service. Newborn screening is the process of testing newborn babies for treatable genetic, endocrinologic, metabolic and hematologic diseases using blood samples on filter paper obtained by pricking a newborn baby’s heel on the second day of life to get a few drops of blood. The development of tandem mass spectrometry expanded newborn screening led to a large expansion of potentially detectable congenital metabolic diseases that affect blood levels of organic acids.

Sample collection for metabolic tests

To keep in context of this review, sample collection details for important parameters are discussed first.

(1) Electrolytes, liver function test (LFT), renal function test (RFT), complete blood count (CBC), glucose

For LFT and RFT 1 ml of plasma or serum sample is collected. Sample should be stored at 2-8°C if the test is to be performed later.

For CBC one 3 ml of blood is collected in vials having anticoagulants like EDTA or K$_2$EDTA.

Glucose: 1 ml plasma or serum is collected and stored at 2-8°C if test is to be performed later. For cases suspected with glycosuria random urine specimens are acceptable, but have no reference intervals.

For electrolytes 24-hour or random urine is accepted. Specimen must be refrigerated if the test is to be performed later.

(2) Collection of plasma for ammonia estimation

It is preferable to collect this sample after at least 6 hours of fasting. Heparin is the preferred anticoagulant, because it has been shown to reduce red blood cell ammonia production. EDTA can also be used. Donors’ arms should be as
relaxed as possible, because muscle exertion may increase venous ammonia levels. Blood is drawn into a chilled, heparinized vacuum tube that is immediately placed on ice, and plasma is separated within 15 minutes. It is crucial to keep blood samples cold after collection, because the ammonia concentration of standing blood and plasma increases spontaneously. Plasma ammonia levels of whole blood maintained at 4°C are stable for <1 hour. When promptly separated from blood, plasma ammonia levels are stable at 4°C for 4 hours and for 24 hours if stored frozen at –20°C.

(3) Collection of blood or cerebrospinal fluid (CSF) for lactate and pyruvate assays

They are measured enzymatically in blood or CSF as an index of impaired pyruvate metabolism due to defects of glucose oxidation or gluconeogenesis. The ratio of lactate to pyruvate reflects the NAD/NADH ratio and is useful in distinguishing primary defects of pyruvate metabolism from defects of electron transport (or oxidation).

Collect venous or arterial blood without prolonged stress to the patient with brief use of the tourniquet, if needed. If collecting blood for several purposes/tests, quickly draw all the blood needed and place initially in a plain tube. Deproteinize immediately to avoid artifacts of lactate formation in red cells or loss of pyruvate.

For this blood or CSF obtained is measured and transferred immediately into a tightly stoppered tube containing 8% w/v perchloric acid. Stopper the tube and shake vigorously for at least 15 seconds. The sample is now stable for local transport to a laboratory where it can be centrifuged. Two centrifugations may be required to obtain a clear supernatant. The supernatant should be removed with a Pasteur pipette and transferred to a tightly stoppered polypropylene tube. Freeze the supernatant, and pack in sufficient dry ice for shipment/transport. Perchloric acid is prepared by mixing 7 ml of 70% perchloric acid and distilled water to make 100 ml total volume. Refrigerate until ready to use.

(4) Collection of plasma for amino acids

Collect 1 ml blood in an EDTA tube (lavender top), mix gently, and centrifuge. Transfer plasma into a polypropylene tube, and freeze until assayed.

Quantitation of amino acids with the Automated Analyzer or Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) has been the method of choice for quick, reliable and effective interpretation of the aminoacidogram in suspected cases of metabolic defects. Several trivial factors when not considered lead to significant deviations from the actual amino acid picture. A few of them are discussed briefly,

(a) Choice of anticoagulant: Heparin has been the most commonly used and preferred anticoagulant for preparation of plasma for amino acid analysis. The mucosin polysaccharide inhibits the formation of thrombin from prothrombin. Normal concentration of use for analysis is 2mg/10ml blood. However, its indiscriminate use leads to hemolysis and may introduce artifacts. Another anticoagulant, EDTA (ethylene diamine tetra acetate) chelates calcium ions for anticoagulant effect and is available in two forms viz. dipotassium and dilithium salt. The former is preferred owing to greater solubility. Ninhydrin positive artifact with heparin or EDTA co-eluting with taurine have been detected.

(b) Hemolysis: Hemolysis can occur during phlebotomy, preparation of the sample and transport of the specimen. Even when hemolysis is not noticeable on naked eye inspection,
spectroscopic examination may reveal bands of oxyhemoglobin. In such samples, increases in concentrations of taurine, glutamic acid and glutathione have been noted and decreases of up to 50\% have been noted with respect to arginine and cystine.\(^9\)

(c) **Deproteinising agent:** Picric acid and sulfosalicylic acid have served the purpose of deproteinising the sample prior to application onto the column. Appreciable losses of tryptophan have been observed with picric acid. Sulfosalicylic acid has the advantage of not altering the amino acid composition and also makes the Dowex treatment that ensues the use of picric acid, unnecessary after deproteinisation.\(^9\)

(d) **Delay in deproteinisation:** Effect of delay in deproteinisation can be readily noticed with reference to cysteine, homocysteine and cystine, wherein they readily bind to red cell and plasma proteins in the time gap available. Conversion of pyruvate to lactate is also favored during the delay. Formation of urea is also consequent to delay, particularly in contaminated specimens. Errors with respect to homocysteine could prove costly when dealing with a homocystinuric on dietary restriction since actual prognosis may not be indicated.\(^9\)

(e) **Buffy coat contamination:** This is probably the easiest contaminant while separating plasma. The effort of acquiring maximum plasma is a great temptation to resist. Contamination with leucocytes and platelets leads to a high estimate of glutamic acid and aspartic acid since these cells contain much higher levels than plasma itself. Other aminoacids are not significantly affected as their concentration in mature erythrocytes is similar to that in plasma. It would be wise enough to leave a 5- 7mm layer above the buffy coat region before aspirating plasma.\(^9\)

(f) **Delay in analysis:** Deproteinised plasma stored for longer periods, even in a freezer, may not result in the most accurate amino acid determinations. Losses in levels of glutamine and asparagine have been noted whilst increases in glutamic acid and aspartic acid have been reported. However, it is generally opined that 90-day freezer storage could still render the sample suitable for amino acid analysis.\(^9\)

Plasma is preferred for aminoacid analysis over serum, which usually stands as the most important sample for a large number of biochemical investigations.

Heparin interferes with detection of sulphur containing aminoacids, cysteine and homocysteine. Thus EDTA sample may be the most accurate for measurement of cysteine concentration.\(^10\)

**Collection of plasma for homocysteine estimation**

Fasting specimen is preferred. Plasma or serum must be separated from cells within one hour of collection. If specimen cannot be separated from the cells within an hour specimen should be stored in ice. EDTA or K\(_2\)EDTA is the preferred anti-coagulant.\(^9\)

(5) **Collection of plasma for carnitine, β-hydroxybutyrate / acetoacetate and free fatty acid assays**

These compounds are measured in plasma as parameters of lipolysis and fatty acid oxidation (e.g. for diagnostic testing of fasting hypoglycemia or after an oral fat tolerance test) or to monitor efficacy of ketogenic diets. The ratio of \(\alpha\)-hydroxybutyrate/ acetoacetate reflects mitochondrial NAD/NADH, and may be a useful parameter for diagnosis of defects of the Krebs cycle.
Collect 1 ml blood is in an EDTA tube (lavender top), mix gently, and centrifuge. Transfer plasma into a polypropylene tube, and freeze if test is to be performed later.

(6) Collection of urine for carnitine or organic acid assays

Carnitine screening is recommended for detection of primary or secondary carnitine deficiency and monitoring of patients being treated with carnitine. Candidates include patients with failure to thrive, cardiomyopathy, weakness, or possible metabolic disorders.

The patient’s perineal area should be thoroughly washed and rinsed with water to remove all dirt, oils, and soap. Collect urine in a thoroughly clean container, preferably cultured container. Transfer 5 ml of urine (minimum 1 ml) to a tightly stoppered polypropylene tube and freeze. Preservatives may be required while collecting urine for certain substances (Table 1).

Metabolic decompensation, such as lactic acidosis, ketosis, or liver failure, gives rise to an abnormal excretion of organic acids (keto branched, dicarboxylic, or aromatic acids, respectively) that are otherwise involved in particular IEM; this sometimes renders interpretation even more difficult. Poor preservation of urine samples will lead to non-enzymatic conversion of all ketoacids to the respective hydroxyacid; for example, acetoxacetate is converted to 3-hydroxybutyrate, and 2-ketoglutarate is converted to 2-hydroxyglutarate.

(7) Collection of urine for mucopolysaccharides (MPS): 5 ml of urine is collected and frozen if test is to be performed later. Morning void is preferred.

(8) Collection of urine for GC-MS: 10 ml of urine is collected and kept in refrigerator.

(9) Collection of whole blood for lymphocyte assays

The lymphocytes must be isolated within 48 hours of collection. Blood must be collected in Anticoagulant Citrate Dextrose (ACD) using sterile technique. 5 ml of blood is preferred for patients < 6 months of age and 10 ml for patients for > 6 months of age.

(10) Collection of blood spots for newborn screening (NBS) and tandem mass spectrometry (TMS)

Heel prick samples must be collected on special filter paper meant for newborn screening analysis and TMS for carnitines and acyl carnitines. For newborn screening collection of

<table>
<thead>
<tr>
<th>Test</th>
<th>Recommended preservative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine organic acids</td>
<td>No additive</td>
</tr>
<tr>
<td>Urine for mucopolysaccharides</td>
<td>No additive</td>
</tr>
<tr>
<td>Urine for Carnitine</td>
<td>No additive</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>25 ml 6N HCl/ 15 gm Boric acid</td>
</tr>
<tr>
<td>Aminolevulonic acid</td>
<td>No additive</td>
</tr>
<tr>
<td>Catecholamines</td>
<td>25 ml 6N HCl/ 15 gm Boric acid</td>
</tr>
<tr>
<td>Cystine</td>
<td>No additive/25 ml 6N HCl/ 15 gm Boric acid</td>
</tr>
<tr>
<td>Metanephrine</td>
<td>25 ml 6N HCl/ 15 gm Boric acid</td>
</tr>
<tr>
<td>Porphobilinogen</td>
<td>No additive</td>
</tr>
<tr>
<td>Porphyrins</td>
<td>5 gm Sodium Carbonate (Refrigerate and protect from light)</td>
</tr>
</tbody>
</table>
A urine sample is erroneous. Dried Blood spot is the preferred sample source for screening newborns by TMS and/or Enzyme Immunoassay (EIA) or Fluorescence based Immuno Assay (FIA). Urine may not be the right choice, since analytes vary in concentration over a large range in newborns. This introduces error in reporting actual levels. Artifacts could also cause a problem in interpretation. GC-MS through which urine may be screened is not a method of choice for NBS due to its low throughput coupled with the various limiting factors associated with urine as a sample for newborn screening. TMS is an FDA approved mode of NBS along with Enzyme Immunoassay (EIA)/ Fluorescence Immunoassay (FIA).

The analysis includes a quantitative High Performance Liquid Chromatography-Mass Spectrometry-Mass Spectrometry (HPLC -MS MS) determination of over 35 different acylcarnitines in plasma, urine, and tissue samples, including short, medium and long-chain derivatives. The acylcarnitine profile includes quantitation of free and total carnitine.

Semi-quantitative determination of over 50 non-volatile organic acids as TMS derivatives can be made by capillary dual column gas chromatography with confirmation by mass spectrometry. It is useful for newborn screening, follow up, and diagnosis and monitoring of a large variety of metabolic disorders, including defects of pyruvate metabolism, the Krebs cycle, amino acid, fatty acid oxidation and the electron transport chain.

Interpretations

The diagnostic specificity of analysis under acute versus asymptomatic conditions may vary considerably. Identification of all relevant organic acids or compounds must be listed and quantity may be mentioned. When no significant abnormalities are detected, an analysis should be reported and interpreted in qualitative terms only. When abnormal results are detected, a detailed interpretation must include; an overview of the results and their significance in perspective of the index case, a correlation to available clinical information, elements of differential diagnosis, recommendations for additional biochemical testing and in vitro confirmatory studies such as an enzyme assay or a molecular analysis. Age dependency of urinary metabolites must be recognized and must be applied during interpretation.

Common pitfalls that may lead to erroneous interpretations include liver disease which may cause aminoacid elevations, renal tubular defects causing generalized aminoaciduria and hypoperfusion leading to lactic acidosis. Drugs such as valproate may cause ketonuria, topiramate and acetozolamide may cause lactic academia. In essence, a single negative test may not exclude an IEM in all cases, thus underlining the significance of collecting a sample during an episode.

A confirmatory assay is always essential since mere presence of an abnormal metabolite might not be indicative of the disorder always.

Conclusion

Samples for inherited metabolic disorders should be collected as mentioned using specific preservatives. For both blood as well as urine there is a minimum volume which should be collected for complete assay. Samples collected during acute episodes could be ‘precious’ and yield more information while screening for an IEM. For newborn screening it should preferably be heel prick blood spot. Urine as a sample for newborn screening is not acceptable for reasons discussed earlier in this review. Clinicians and laboratory personnel should have a clear view
on the samples to be collected for accurate results. In general it may be accepted that EDTA plasma is a preferred choice for aminoacid and carnitine estimations. Heparinized plasma is the preferred choice for most enzyme assays with few exceptions. Urine is the preferred choice for analysis of organic acids, MPS, porphyrins, sugars and metachromatin granules. CSF is preferred for analysis of glycine and neurotransmitters. One must remember the fact that “Results of a metabolic investigation are as good as a sample”.

Points to Remember

• An appropriate sample according to the metabolite of interest is to be collected, processed and analysed as per standard protocol.

• While interpreting the result, factors influencing the result like whether sample has been collected during symptomatic or asymptomatic period, co morbid are conditions, etc. are to be considered.

References


ERRATUM

In the previous issue, “Indian Journal of Practical Pediatrics” Vol.12 No.1 (Jan – Mar), 2010 page no.7, 2nd para, in the second line, to read as “sensitive methods like PCR” instead of “sensitive methods like PC31”.
INBORN ERRORS OF METABOLISM IN INFANCY AND CHILDHOOD PRESENTING WITH METABOLIC ACIDOSIS

* Prasad C
** Rupar CA

Abstract: Disturbances of acid-base homeostasis are not uncommon. These are important indicators of underlying disease in infants and children. Metabolic acidosis is one of the most common perturbations noted in acute pediatric emergencies. Frequent causes of metabolic acidosis include diabetic ketoacidosis, shock and tissue hypoxia, salicylate and ethanol poisoning. However, it is important to recognize that many inborn errors of metabolism (IEM) such as organic acidemias and primary lactic acidosis also present with a persistent metabolic acidosis. Calculation of the anion gap, presence or absence of hyperammonemia, hypoglycemia and ketosis are essential in the diagnosis of these patients. Early diagnosis and appropriate management is necessary to optimize the outcome. IEM have a genetic basis and appropriate genetic counseling needs to be provided to the families.

Key words: Organic acidemias, Lactic acidosis, Hypoglycemia, Ketosis.

Metabolic acidosis is characterized by decrease in arterial blood pH (pH< 7.30), plasma bicarbonate and pCO₂. It can result from an abnormal loss of bicarbonate or an accumulation of acid and can occur with a normal (10-15 mEq/L) or an increased anion gap (>15 mEq/L) that is measured as the difference between plasma [Na⁺] and the sum of plasma [Cl⁻] and [HCO₃⁻]. An increased anion gap can result from the excess production of acid that occurs in diabetic ketoacidosis, primary or secondary lactic acidemias and several IEM.¹⁻⁴ Metabolic acidosis with a normal anion gap is almost always hyperchloremic, caused by renal or gastrointestinal loss of bicarbonate (Fig.1). This review briefly outlines the pathogenesis, clinical presentations and approaches to diagnosis and management of IEM that present with metabolic acidosis. Treatment of metabolic acidosis is primarily based on the identification of the underlying cause.

Biochemical abnormalities

Routine laboratory tests including blood glucose, urine ketones, ammonia and lactate can be very helpful in establishing a differential diagnosis of IEM that present with metabolic acidosis. For example, neonates have the capacity to rapidly metabolize fat to produce ketones and the presence of urine ketones in infants less than a month should raise suspicion of organic acidemias. Hypoglycemia with metabolic acidosis can be present in metabolic disorders such as glycogen storage disease type 1, fatty acid β-oxidation disorders and disorders of gluconeogenesis. Interestingly
organic acidemias can present with hypo or hyperglycemia.

Lactic acidosis is the most common metabolic acidosis. (Fig.2) It can be caused by shock or poor perfusion from a variety of causes including congenital heart defects (for example coarctation of the aorta and hypoplastic left heart syndrome). Lactate levels less than 10 mmol/L may not result in an elevated anion gap however high anion gap metabolic acidosis can be due to lactic acidosis (levels>10-15mmol/L). There are primary lactic acidosis disorders such as pyruvate dehydrogenase deficiency, pyruvate carboxylase deficiency and respiratory chain disorders. Elevations in lactate reflect increased concentrations of pyruvate, the assay of which is not easily available in most laboratories. Accumulated pyruvate, is also converted to lactate and alanine. The ratio of plasma lactate concentration to pyruvate reflects the redox potential within the cytosol. A decreased lactate/pyruvate ratio of <10 indicates pyruvate dehydrogenase (PDH) deficiency and an increased lactate/pyruvate ratio (>25) is
suggestive of pyruvate carboxylase deficiency or mitochondrial respiratory chain abnormalities. Secondary lactic acidosis is also a feature of organic acidemias and fatty acid β-oxidation disorders.

Hyperammonemia and metabolic acidosis can occur both in fatty acid oxidation disorders or organic acidemias. Hyperammonemia usually occurs as a result of secondary inhibition of the urea cycle enzymes by the toxic metabolites. Although assays for lactate and ammonia are technically straightforward, the concentrations of both of these metabolites can be elevated misleadingly by improper specimen collection and transportation to the laboratory.

Approach to diagnosis

Clinical features

IEM with metabolic acidosis can present from the newborn period throughout childhood. The neonatal period is one of the more vulnerable ages and many of the IEM with metabolic acidosis present during this time. The neonate has limited clinical symptoms that are also common to other disorders such as infection, intracranial hemorrhage or other newborn
emergencies. These include poor sucking, hypotonia, lethargy, seizures, abnormal movements, bleeding, hypothermia, apnea and vomiting. The presence of unusual odors such as the smell of sweaty feet in isovaleric acidemia, maple syrup in maple syrup urine disease and cat urine in 3 methyl-crotonyl carboxylase deficiency is helpful. In later periods in life intermittent periods of ataxia, encephalopathies and neurological presentations along with “Reye syndrome” like phenotype may be associated with metabolic acidosis.

A detailed family history is essential to identify consanguinity as most of the IEM presenting with metabolic acidosis are inherited in an autosomal recessive manner. The most prominent exceptions are the E1-α subunit form of pyruvate dehydrogenase deficiency (primary lactic acidemia) which is inherited in an X-linked manner. Another exception is the maternal inheritance pattern seen in mitochondrial disorders such as MELAS (due to mitochondrial DNA point mutations). Similarly, knowledge of ethnic background is helpful as some of these disorders are more common in particular ethnic groups. Enquires should also identify any history of unexplained neonatal or infant deaths, unexplained mental retardation or children with similar disease. Most affected infants are born at full term with a normal birth weight. A detailed examination focusing on vital signs, the presence or absence of facial dysmorphism, organomegaly and detailed neurological examination to look for hypo/hypertonia, abnormal movements, and presence of petechiae or bleeding should be carried out systematically.

Investigations

Investigative work-up in cases with metabolic acidosis should include the preliminary and then the specific tests (Table 1). For best results, it is helpful to provide adequate clinical information to the laboratory and to be familiar with the laboratories reference ranges.

Specific IEM disorders to consider with metabolic acidosis

Although the list of conditions presenting with metabolic acidosis is quite long, the disorders are grouped into the following major categories.

1. Organic acidemias
2. Fatty acid β-oxidation disorders
3. Lactic acidoses

1. Organic acidemias

A number of these disorders are due to defects in the catabolic pathways for the branched chain amino acids or other amino acids after deamination. Examples of organic acidemias include methylmalonic acidemia (MMA), propionic acidemia (PA) and isovaleric acidemia (IVA). These disorders may present in the neonatal period with an increased anion gap metabolic acidosis. The most severe presentation is a healthy newborn infant who becomes very ill within the first 24-48 hours with profound metabolic acidosis, hyperammonemia, urine ketosis and encephalopathy. Features of cerebral edema with a bulging fontanel may be present. Neutropenia and thrombocytopenia with elevations in uric acid are noted. Neurological abnormalities worsen with abnormal movements, seizures and alterations in tone. EEG may show burst suppression pattern.

The organic acidemias (IVA, PA and MMA) commonly feature dehydration, moderate hepatomegaly and increased anion gap metabolic acidosis and ketonuria. Presence of hyperammonemia can induce respiratory alkalosis or a mixed form of acid base disorder abnormality which can lead to a false diagnosis of urea cycle
Table 1. Recommended investigations for metabolic disorders

<table>
<thead>
<tr>
<th>Preliminary Tests</th>
<th>Specialized Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose</td>
<td>Urine organic acids by gas chromatography-mass spectrometry (GC-MS)</td>
</tr>
<tr>
<td>Electrolytes</td>
<td>Plasma amino acids</td>
</tr>
<tr>
<td>Blood gases</td>
<td>Plasma carnitine (acyl and free)</td>
</tr>
<tr>
<td>Calculate anion gap</td>
<td>Plasma acylcarnitine profile</td>
</tr>
<tr>
<td>Ammonia</td>
<td>Skin Fibroblasts to measure specific enzyme assays for specific disorders</td>
</tr>
<tr>
<td>Lactate</td>
<td>DNA based studies for molecular analysis</td>
</tr>
<tr>
<td>Urine for ketones</td>
<td>Lactate/Pyruvate ratio</td>
</tr>
<tr>
<td>Uric acid</td>
<td>CSF lactate, amino acids</td>
</tr>
<tr>
<td>Complete blood count for</td>
<td>Muscle biopsy for electron microscopy and respiratory chain analysis</td>
</tr>
<tr>
<td>pancytopenia, neutropenia</td>
<td></td>
</tr>
<tr>
<td>thrombocytopenia</td>
<td></td>
</tr>
</tbody>
</table>

disorder. Hypo or hyperglycemia with ketosis can mimic neonatal diabetes. In some cases combination of vomiting, abdominal distension and constipation may suggest gastro-intestinal obstruction such as pyloric stenosis. Without prompt management, these neonates can succumb to their illness. The clinical features of these diseases tend to be non-specific with considerable overlap among the disorders. Definitive diagnosis is dependent on the results of laboratory investigations with the key tests being plasma amino acids, urine organic acids and plasma acylcarnitine profile with expert interpretation.

Maple syrup urine disease (MSUD) presents as an encephalopathy usually without a prominent metabolic acidosis. The presentation of MSUD in infants overlaps sufficiently with the organic acidemias with metabolic acidosis that it is relevant to discuss with the other disorders. Maple syrup urine disease is characterized by significant increases in the plasma levels of the branched chain amino acids, valine, leucine and isoleucine along with the pathognomonic alloisoleucine. The corresponding 2-keto acids are present in the urine and can be identified by 2, 4 dinitrophenylhydrazine (DNPH) test as well as urine organic acid analysis.

Along with the acute infantile presentations there can be acute late onset forms where the disease may present after initial symptom-free period in childhood, adolescence or adulthood. Onset of an acute attack can be precipitated by stress, infection, increased protein intake, constipation or for no overt reason. Most of the late onset presentations are neurologic (recurrent episodes of ataxia, coma, seizures, developmental delay and hypotonia) or gastro-intestinal (recurrent episodes of vomiting, failure to thrive and pancreatitis-leading to hyperglycemia and hypocalcemia). Other rare presentations are skin disorders resembling staphylococcal scalded skin syndrome or acrodermatitis enteropathica.
Renal complications are commonly seen with MMA and can include renal tubular acidosis or tubulo-interstitial nephritis in older children and adults with MMA. Other organic acidemias that can present with metabolic acidosis include 3-hydroxy-3 methylglutaryl-CoA lyase (HMG CoA lyase deficiency) that presents with hypoketotic hypoglycemia and shares many features with fatty acid oxidation disorders such as medium chain acyl-coA dehydrogenase deficiency (MCAD deficiency). Other rare causes of metabolic acidosis are ketolytic disorders such as Succinyl-CoA-3 Oxoacid CoA- Transferase deficiency (SCOT) and thiolase deficiency and pyroglutamic aciduria (hemolytic anemia, metabolic acidosis, CNS damage and recurrent bacterial infections). All the above disorders are inherited in an autosomal recessive manner.

2. Fatty acid β-oxidation disorders

The disorders include very long chain acyl-CoA dehydrogenase (VLCAD) deficiency, medium chain acyl-CoA dehydrogenase (MCAD) deficiency, carnitine transporter deficiency and carnitine palmitoyl transferase 1 (CPT1A) deficiency among others. Neonatal presentations can occur with VLCAD and carnitine transporter deficiency. These two disorders and other fatty acid β-oxidation disorders can present in children or adults. The common clinical phenotype is of intermittent metabolic acidosis and hypoketotic hypoglycemia. A low 3-hydroxybutyrate in plasma at the time of hypoglycemia is suggestive of fatty acid oxidation disorder. Neonatal presentation can include hyperammonemia and mild lactic acidosis. Cardiomyopathy is a feature of carnitine transporter deficiency and VLCAD while MCAD and CPT1A deficiency present with mild hepatomegaly, hypoglycemia and encephalopathy (Reye syndrome-like phenotype). There is a nearly 25 % mortality at the initial presentation of unrecognized MCAD deficiency thus making a strong case for newborn screening for these set of disorders. The above disorders are also inherited in an autosomal recessive manner.

3. Lactic acidosis

Patients with IEM presenting with a primary lactic acidosis are divided into the following two categories:

a) Defects in gluconeogenesis (glycogen storage disease type 1, hereditary fructose intolerance, phosphoenolpyruvate carboxykinase deficiency, fructose -1, 6, diphosphatase deficiency). In these disorders, hypoglycemia and lactic acidosis are associated with hepatomegaly and hyperuricemia.

b) Defects in pyruvate metabolism pyruvate carboxylase (PC), pyruvate dehydrogenase (PDH) deficiency, mitochondrial electron transport chain disorders. Lactic acidemia is the most significant metabolic abnormality in the disorders of pyruvate metabolism. Pyruvate carboxylase deficiency can be considered as a gluconeogenic disorder as well as a disorder in pyruvate metabolism, however it is discussed as a disorder of pyruvate metabolism since lactic acidosis is usually much more prominent than hypoglycemia.

Pyruvate carboxylase deficiency presents usually in infancy as a severe and persistent lactic acidosis with significant neurological involvement. It is inherited in an autosomal recessive manner and has a poor prognosis even with early identification of affected patients. Pyruvate dehydrogenase is a complex enzyme that has four subunits encoded by five genes that convert pyruvate to acetyl-CoA for entry into the tricarboxylic acid cycle and ultimately provides energy for oxidative phosphorylation. The most common type of PDH deficiency is E1α deficiency that is inherited in an X-linked
manner (as mentioned above) but females are often affected. The remaining four types of PDH deficiency are autosomal recessively inherited. Deficiency of PDH is a relatively frequent cause of Leigh’s encephalopathy and neonatal lactic acidosis. Mid-facial hypoplasia (similar to that seen in fetal alcohol syndrome) and microcephaly are often present. Cranial MRI may show abnormalities in neural development particularly agenesis of corpus callosum. In PDH deficiency the lactic acidosis worsens with increasing carbohydrate intake as pyruvate is converted to lactate instead of acetyl CoA with a normal lactate/pyruvate ratio. Fasting hypoglycemia is not usually seen with PDH deficiency.

Management of infants and children diagnosed/suspected of having metabolic acidosis caused by IEM

General

Before starting emergency therapy additional blood and urine samples should be obtained for specific investigations. **Blood:** 4-5 ml in lithium heparin should be centrifuged rapidly and plasma stored frozen at -20° C and **Urine:** (4-5 ml should be stored at -20° C). Initial management involves stabilization of the sick neonate including use of ventilatory and cardiac support. The goal of the treatment is to minimize catabolism and remove toxic metabolites. These measures can be started before the final diagnosis is established. Promptness in diagnosis and timely initiation of appropriate management are major determinants of the ultimate clinical outcome. In the organic acidemias and fatty acid β-oxidation disorders adequate glucose should be provided. Higher rates of glucose infusion require access through a central line and with frequent monitoring. Insulin can be administered if glucose is >12 mmol/L (216 mg/dL) and glycosuria occurs. Acidosis should be corrected by using bicarbonate 1mEq/kg given gradually. Electrolyte disturbances, dehydration and hypothermia should be corrected. Abnormal metabolites that are characteristic of specific organic acidemias may accumulate and in some disorders hyperammonemia may occur. These may impair neurological functions and should be removed by dialysis. Hemodialysis or hemofiltration are very useful but availability of hemodialysis and ability to place an adequate size catheter in a small infant may be a challenge. Fluid balance should be carefully maintained. The use of urea cycle cocktail is generally not effective in the management of hyperammonemia of organic acidemias. In the primary lactic acidemias, hypoglycemia should be identified, corrected and monitored. However the management principle for PDH deficiency is to use lipid as the primary source of energy rather than carbohydrate and bypass PDH to provide acetyl-CoA.

**Medications**

Carnitine (100-400 mg/kg) intravenously divided into three doses can be instituted prior to the definitive diagnosis (Table 2). Organic acids are excreted in part as acylcarnitines often resulting in low free plasma carnitine levels in organic acidemias. In isovaleric acidemia glycine plays an important role in promoting metabolite excretion. This can be used in conjunction with carnitine. Carnitine therapy for fatty acid oxidation disorders particularly VLCAD remains controversial in view of risk of cardiac arrhythmias, however carnitine has been used in MCAD deficiency and is the mainstay of treatment of carnitine transporter deficiency. Pharmacological doses of vitamin B12 for methylmalonic acidemia are useful in vitamin responsive methylmalonic acidemias. Similarly biotin should be given for biotinidase and holocarboxylase synthetase deficiency. Dichloroacetate has been used to reduce lactic
acid concentration but does not affect the neurological outcome.\textsuperscript{8} Use of carbaglu (N-acetyl-glutamate, the natural activator of carbomyl phosphate synthetase\textsuperscript{1} is being explored in hyperammonemia associated with organic acidemia.\textsuperscript{9}

**Nutrition**

Dietary treatment of most organic acidemias requires protein restriction that should be implemented as part of a complete nutritional management program under the supervision of a dietitian to avoid nutritional deficiencies and promote adequate growth. It is important to note that vitamin responsive IEM are usually less severe than the non-vitamin–responsive variants. Ongoing monitoring for trace elements deficiency should be carried out in patients on modified diets. Bone health is also becoming a major issue with number of metabolic disorders due to altered mineral status, presence of metabolic acidosis and accumulation of toxic compounds. A regular bone density measurement along with appropriate supplementation of calcium and vitamin D is useful for these patients.

**Ongoing management**

These emergency measures may cause inadequate nutrition if used for a prolonged period and exacerbate catabolism leading to frank malnutrition. Proper assessment of growth and anthropometry is an essential part of management. Periodic neurodevelopmental assessments are recommended as in some of these patients hyperammonemia can cause neurological damage. A number of these children with severe metabolic disorders may die despite the heroic measures. Post mortem metabolic autopsy should be carried out\textsuperscript{10} (Table 3).

**Recent advances**

With advent of newborn screening using tandem mass spectrometry, many of these disorders can be diagnosed pre-symptomatically in the neonatal period. This requires public health programs that include centralized laboratories, efficient communication systems and appropriately trained physicians, nurses and dietitians to identify and retrieve screen positive infants to the clinic in a timely manner. However, even with newborn screening, babies at the severe end of the clinical spectrum of some of the organic acidemias will present before the results of newborn screening testing are available. Pediatricians and neonatologists need to consider these disorders in newborn.\textsuperscript{11,12} Prenatal diagnosis in future pregnancies can be established more reliably by molecular diagnostic testing than through enzymatic studies for several IEMs. With improvements in technology and clinical

**Table 2. Suggested management of suspected organic acidemias**

<table>
<thead>
<tr>
<th>Treatment</th>
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<tbody>
<tr>
<td>High caloric, protein free nutrition (as mentioned above with the help of dietician)</td>
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<tr>
<td>Insulin if required</td>
</tr>
<tr>
<td>Hydroxycobalamin (Vitamin B\textsubscript{12}, 1-2 mg/day IV; Cyanocobalamin can be used if hydroxycobalamin is not available. However it may not be as effective) (For MMA)</td>
</tr>
<tr>
<td>Biotin 10-20mg/day IV or oral (For biotinidase deficiency, multiple carboxylase deficiency)</td>
</tr>
<tr>
<td>Thiamine 10-50 mg/day IV or oral in 1-2 doses (For MSUD)</td>
</tr>
<tr>
<td>Riboflavin 20-50 mg/day oral</td>
</tr>
<tr>
<td>Carnitine 100-400 mg/kg IV in 3 doses (For propionic and methylmalonic acidemia)</td>
</tr>
</tbody>
</table>
Table 3. Peri-mortem/post-mortem metabolic autopsy specimens

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Storage/Handling Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>4-5 ml (Lithium Heparin tube) Heparinized, separated and frozen at -20°C</td>
</tr>
<tr>
<td>Blood</td>
<td>EDTA tube for DNA banking</td>
</tr>
<tr>
<td>Filter paper</td>
<td>For acylcarnitine analysis</td>
</tr>
<tr>
<td>blood spots</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>4-5 ml frozen and stored at -20° C</td>
</tr>
<tr>
<td>Skin</td>
<td>Fibroblast culture in Hanks solution stored at 4-8° C (not frozen) for enzyme studies</td>
</tr>
<tr>
<td>Liver</td>
<td>Frozen for enzymology</td>
</tr>
<tr>
<td>Muscle</td>
<td>Frozen for histochemistry and enzymology</td>
</tr>
</tbody>
</table>

awareness more babies born with IEM are surviving than previously. Long term follow up is essential to identify and treat late complications that can occur such as renal complications in MMA. Families affected with these disorders need support and appropriate genetic counselling. Parent support groups are of great help in this direction.

Points to Remember

- **Presence of metabolic acidosis particularly with high anion gap should alert the pediatrician to a possibility of inborn errors of metabolism.**

- **Inborn errors of metabolism can mimic common neonatal conditions such as sepsis and vice versa.**

- **Biochemical tests such as ammonia levels, presence or absence of ketones and lactate measurements can help with the diagnosis of inborn errors of metabolism.**

- **Prompt and early diagnosis is essential as many of these inborn errors of metabolism are treatable.**

- **Early treatment optimizes the neurocognitive development of the infant.**

- **Inborn errors of metabolism have a genetic basis in most instances so establishing a diagnosis will also help in genetic counselling and family screening.**

Acknowledgement

We would like to thank all the patients, families, residents and students who have inspired us to a better understanding of these groups of disorders. Chitra Prasad would like to specially thank Dr. Mark Korson (Director of Metabolic Program, Tufts University New England Medical Centre, Boston) for his teaching and approach to metabolic disorders.

References


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**NEWS AND NOTES**

**FIRST MID TERM NATIONAL CME OF IAP RESPIRATORY CHAPTER**

Venue: B.R.Singh Hospital for Medical Education & Research, Sealdah, Kolkata.

at 9. 30 am – 5 pm on 6th June, 2010, Sunday

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RECURRENT HYPOGLYCEMIA
AND INBORN ERRORS OF
METABOLISM

* Madhulika Kabra
** Neerja Gupta

Abstract: Many of the inborn errors of metabolism, including urea cycle defects, organic acidemias, and certain disorders of amino acid metabolism, present in the young infant with symptoms of an acute or chronic metabolic encephalopathy. Typical symptoms include lethargy, poor feeding, apnea or tachypnea, recurrent vomiting, metabolic acidosis and/or hyperammonemia. Hypoglycemia may be the predominant finding in a number of inborn errors of metabolism, including glycogen storage disorders, defects in gluconeogenesis, and fatty acid oxidation defects.

Keywords: Hypoglycemia, Inborn errors of metabolism, Glycogen storage disorders, Fatty acid oxidation defects.

Within a few days or weeks after birth, a previously healthy neonate may begin to show signs of an underlying metabolic disorder. Although the clinical picture may vary, infants with metabolic disorders typically present with lethargy, decreased feeding, vomiting, tachypnea (from acidosis), decreased perfusion, and seizures. As the metabolic illness progresses, there may be increasing stupor or coma associated with progressive abnormalities of tone (hypotonia, hypertonia), posture (fisting, opisthotonus), and movements (tongue-thrusting, lip-smacking, myoclonic jerks), and with sleep apnea. Metabolic screening tests should be initiated. Elevated plasma ammonia levels, hypoglycemia, and metabolic acidosis, if present, are suggestive of inborn errors of metabolism.¹

This article will focus primarily on inborn errors of metabolism causing recurrent hypoglycemia.

Hypoglycemia (blood sugar<45mg/dl at all ages) is a common nonspecific problem in severely ill neonates and young infants, regardless of the cause of the illness. Unless the cause of the symptoms is recognized and treated, this is followed by disturbance of consciousness with drowsiness progressing rapidly to stupor and coma accompanied by convulsions. In very young infants, the early signs may be subtle with nothing more than irritability, sweating and somnolence. A seizure may be the first recognized indication of the problem. Regardless of the cause, correction of hypoglycemia without delay is at least as important as making a specific diagnosis. The differential diagnosis of hypoglycemia is made easier by some understanding of the normal mechanisms for maintaining normal plasma glucose concentrations during fasting.

During the intervals between meals, the plasma glucose concentration is maintained by two general mechanisms.²

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** Senior Research Officer

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Mechanisms directed at producing glucose (glycogen breakdown and gluconeogenesis)

Mechanisms that decrease peripheral glucose utilization by providing alternative energy substrate (fatty acid and ketone oxidation)

Hence, hypoglycemia can occur as a result of primary or secondary defects in glucose production (deficiency of supply), or as a result of defects in fatty acid or ketone oxidation (over utilization). Hypoglycemia and its associated symptoms occasionally may be seen in infants with disorders of protein intolerance, but it is seen more commonly in disorders of carbohydrate metabolism or fatty acid oxidation.

**Inborn errors of metabolism causing hypoglycemia**

A. Primary defects in glucose production (Gluconeogenesis and glycogenolysis disorders)

- Glucose-6-phosphatase deficiency (GSD1a)
- Glucose 6-phosphate translocase deficiency (GSD1b)
- Glycogen synthase deficiency (GSD0)
- Amylo 1,6 glucosidase (debranching enzyme) deficiency (GSD3)
- Liver phosphorylase deficiency (GSD 6)
- Phosphorylase kinase deficiency (GSD 9)
- Phosphoenolpyruvate carboxykinase (PEPCK) deficiency (rare)
- Fructose 1, 6 diphosphatase deficiency
- Pyruvate carboxylase deficiency
- Galactosemia
- Hereditary fructose intolerance

B. Over utilization of glucose

(i) Primary or secondary defect in fatty acid oxidation

Carnitine transporter deficiency (primary carnitine deficiency)
Carnitine palmitoyl transferase 1 deficiency
Carnitine translocase deficiency
Carnitine palmitoyl-transferase 2 deficiency
Secondary Carnitine deficiencies
Very long/long/medium/short chain acyl CoA dehydrogenase deficiency

(ii) Hyperinsulinism

C. Others

Aminoacid and organic acid disorders

A) Primary defects in glucose production (Gluconeogenesis and glycogenolysis disorders)

Hypoglycemia with permanent hepatomegaly is mostly due to inborn error of metabolism. Acquired or inherited hepatomegaly associated with severe liver failure in early or late infancy or childhood can give rise to severe hypoglycemia, which appears after 2-3 hour of fasting and causes moderate lactic acidosis but no ketosis. Among the best known inborn errors of metabolism associated with hypoglycemia are the hepatic glycogen storage diseases (GSD). The hypoglycemia in these disorders is related to the inability of the liver to release glucose from glycogen, and it is most profound during periods of fasting. Hypoglycemia, hepatomegaly and lactic acidosis are prominent features of these disorders. The normal physiologic response to decreased glucose production is increased mitochondrial fatty acid beta oxidation and production of ketones (ketotic hypoglycemia). However, in GSD1, ketogenesis is often suppressed and plasma and urinary ketone levels, though elevated, may be inappropriately low for the degree of hypoglycemia. In gluconeogenesis defects involving glucose-6-phosphatase or
fructose diphosphatase, fasting hypoglycemia is classically associated with ketolactic acidosis. In glycogenosis type III and glycogen synthase deficiency, fasting hypoglycemia is associated with hyperketosis but there is no metabolic acidosis and fasting lactic acidemia is low. However presence of significant postprandial hyperlactic acidemia is highly suggestive of these diagnoses.

**GSD1** (von Gierke disease) : It presents with severe hypoglycemia at 3-6 months of age precipitated when intervals between feeds is prolonged, or there is associated intercurrent illness and is often heralded by a seizure or coma. Some infants may have failure to thrive or tachypnea due to lactic acidosis. Affected children are usually pale and pasty looking with characteristic cherubic facies because of the doll like appearance caused by chubby cheeks. Truncal obesity and marked abdominal protuberance contrast with typically thin extremities. Recurrent nose bleeds are common as a result of a secondary defect in platelet function.

**Enzyme involved** : The basic defect in GSD1 is deficiency of the production of glucose from glucose 6 phosphate, the final common pathway for glycogenolysis and gluconeogenesis. The most common variant is type Ia and is caused by deficiency of glucose 6 phosphatase. It is expressed in liver and kidney. The nontype Ia variants are caused by deficiency in the microsomal transport of glucose 6 phosphate (type Ib), phosphate(type Ic) or glucose (type Id). Type Ib and Ic are clinically indistinguishable from type Ia and are associated with persistent neutropenia, and affected children typically have histories of recurrent pyogenic infections and pyorrhea.

**Genetics** : All GSD except some forms of GSD type VI (X linked) are autosomal recessive.

**Investigations** : Blood sugar to look for hypoglycemia. Serial plasma glucose shows that the tolerance to fasting is poor often less than 3 hours. The hypoglycemia is characteristically unresponsive to administration of glucagon. There is a significant rise in plasma lactate in response to glucagon.

- Lactate levels for lactic acidosis
- Hypertriglyceridemia, hyperuricemia, Increased transaminases
- Hypophosphatemia
- The kidneys are typically enlarged and mild renal dysfunction is common
- Liver biopsy shows massive glycogen accumulation including glycogen within the nucleus of hepatocytes. There is marked accumulation of macrovesicular fat, but typically no fibrosis, evidence of biliary obstruction or inflammation.
- Enzyme studies on fresh liver biopsy specimen
- DNA analysis for a specific molecular defect

**Therapy** of all types of GSD I is aimed primarily at preventing hypoglycemia by administration of frequent (every 2-3 hrs) low fat feeds, containing the little fructose (less vegetables and fruits) and lactose (soy based milk replacement). This is supplemented by intermittent ingestion of uncooked cornstarch during the day and tube feeding with formula during the night.

- Calcium supplementation
- Allopurinol if required
- For Type Ib/Ic granulocyte colony stimulating factor (G-CSF) 2-3 μg/kg/d can be given for neutropenia
Monitor

- Blood sugar (>80 mg/dl) - Aim for blood lactate < 1.5 mmol/l, normal triglycerides, uric acid and liver function
- Yearly ultrasound of liver
- Check renal function regularly after 14 years of age

**GSD III** commonly presents as asymptomatic firm and nontender hepatomegaly discovered incidentally on routine physical examination. There may be associated splenomegaly. In most patients hypoglycemia does not occur or occur only after prolonged fasting. In a significant minority of patients it may present as sever infantile form like GSD I. However, it is easily distinguishable by the absence or very mild presence of lactic acidosis and hyperuricemia. There is ketosis during fasting and moderate increase in liver AST and ALT, which as a rule do not occur in GSD I. These patients show rise in plasma glucose in response to ingestion of galactose, fructose indicating that gluconeogenesis is intact. They also show a significant rise in plasma glucose in response to glucagon administration 2-4 hrs after feeding. Liver biopsy shows increased glycogen and variable interlobular fibrosis, but very little fat. Rarely, the fibrosis progress to frank cirrhosis producing portal hypertension and liver failure.

**Fanconi Bickel syndrome (GSD type XI)** is characterized by fasting hypoglycemia, marked hepatomegaly associated with early onset renal tubular dysfunction, characterized by polyuria, hypophosphataemic rickets, hyperchloremic metabolic acidosis and severe growth retardation. This is caused by mutations in GLUT2 gene encoding for the liver type glucose transporter.

The triad of hypoglycemia, marked hepatomegaly and lactic acidosis is also characteristic of other defects of gluconeogenesis such as hereditary fructose intolerance (HFI), fructose 1,6 diphosphatase deficiency, PEPCK deficiency and sometimes PC deficiency. Definitive diagnosis requires measurement of the enzyme in fresh liver biopsy in former two and in the fibroblast predominating in mitochondrial isozyme in latter two. In fructose 1,6 diphosphatase deficiency response to glucagon is preserved.

Hypoglycemia may be a prominent feature of both galactosemia and hereditary fructose intolerance, although symptoms of the latter disorder occur only after fructose (sucrose) has been introduced in the diet. These patients develop clinical symptoms only after intake of lactose (milk or milk products) or fructose or sucrose respectively, in the diet. If galactosemia or fructosemia is suspected, the urine should be tested simultaneously with Benedict’s reagent and with a glucose oxidase method. The glucose oxidase method is specific for glucose, and Benedict’s reagent can detect any reducing substance. A negative dipstick result for glucose with a positive Benedict’s reaction means that a nonglucose reducing substance is present. With appropriate clinical findings, this is most likely to be galactose or fructose. The glycosuria in these conditions typically clears rapidly after removal of toxic sugars from diet. Therefore, a negative test does not eliminate the possibility of one of these disorders, particularly if the patients have been on intravenous glucose for more than a few hours. If the diagnosis of galactosemia is suspected, whether or not reducing substances are found in the urine, galactose-containing feedings should be discontinued immediately and replaced by soy formula or other lactose-free formula pending the results of an appropriate enzyme assay on erythrocytes to confirm the diagnosis.

**Classical Galactosemia**: It is due to deficiency of the enzyme galactose-1-phosphate uridyl
transferase results in an accumulation of galactose-1-phosphate and other metabolites such as galactitol that are thought to have a direct toxic effect on the liver and on other organs. Jaundice and liver dysfunction in this disorder are progressive and usually appear at the end of the first or during the second week of life with vomiting, diarrhea, poor weight gain, and eventually cataract formation. Progression of symptoms occurs after start of milk feeds, usually starting on 3rd or 4th day. The disease may present initially with indirect hyperbilirubinemia resulting from hemolysis secondary to high levels of galactose-1-phosphate in erythrocytes. Alternatively, the effects of acute galactose toxicity on the brain may rarely cause the CNS symptoms to predominate. Untreated infants with galactosemia, if they survive the neonatal period, have persistent liver disease, cataracts, and severe mental retardation. Many affected infants die of Escherichia coli sepsis in the neonatal period, and the early onset of sepsis may alter the presentation of the disorder.

Diagnosis is confirmed by either enzyme analysis or specific mutational analysis.

Therapy- Lactose free, galactose restricted diet throughout life is required.

Hereditary fructose intolerance (HFI): The development of symptoms is clearly related to the ingestion of fructose or sucrose presenting with intractable vomiting and symptomatic hypoglycemia. More prolonged exposure results in failure to thrive, chronic irritability, hepatomegaly, abdominal distension, edema and jaundice. Milder variants of disease are common and may present with bloating, abdominal distension and diarrhea.

Investigations shows presence of hypoglycemia, marked lactic acidosis, hyperuricemia, hypophosphatemia, hepatocellular dysfunction like raised aminotransferases, prolonged prothrombin and partial thromboplastin time, hypoalbuminemia, hyperbilirubinemia and renal tubular dysfunction (hyperchloremic metabolic acidosis, generalized aminoaciduria)

Diagnosis is confirmed by demonstration of deficiency of aldolase B in fresh liver biopsy sample.

B. Over utilization of glucose

Increased glucose utilization (hypoketotic hypoglycemia) occurs as a result of hyperinsulinism or as a result of primary or secondary defect in fatty acid oxidation and are easily distinguished by presence of low free fatty acid in the hyperinsulinism. It is important to discover the timing of hypoglycemia and to search for metabolic acidosis and ketosis when the patient is hypoglycemic. Unpredictable postprandial or very short fasting hypoglycemia (2-6hrs) is mostly due to hyperinsulinism and growth hormone deficiency. Prolonged fasting hypoglycemia (8-24 hrs) is typically seen in fatty acid oxidation defects or systemic carnitine deficiency.

Fatty acid oxidation defects: A number of inherited defects in fatty acid oxidation have been identified in infants presenting with hypoglycemia. These disorders are important because of their apparent frequency and because of the variability of the initial presentation. Affected infants have an impaired capacity to use stored fat for fuel during periods of fasting and readily deplete their glycogen stores. Despite the development of hypoglycemia, acetyl CoA production is diminished, and ketone production is impaired. The hypoglycemia occurring in these conditions is typically characterized as nonketotic, although small amounts of ketones may be produced. Hypoglycemia may occur as an isolated finding or may be accompanied by many of the other biochemical derangements
typically associated with Reye syndrome, such as hyperammonemia, metabolic acidosis, and elevated transaminases. Hepatomegaly may or may not be present. Any infant presenting with findings suggesting Reye syndrome should be evaluated for fatty acid oxidation defects. Because the incidence of true Reye syndrome has decreased, most children presenting at any age with this constellation of findings have an inherited metabolic disorder.

**Medium-chain acyl CoA dehydrogenase deficiency** is the most common of the fatty acid oxidation defects and mostly presents as acute or recurrent Reye-like syndrome: vomiting, lethargy, drowsiness, stupor, seizures, hepatomegaly, hypoglycemia, and hyperammonemia. In addition to presenting as nonketotic hypoglycemia or a Reye’s-like syndrome, it may present as sudden death or an acute life-threatening event. Many reports of infants diagnosed as having medium-chain acyl CoA dehydrogenase deficiency have described a history of a sibling who died of SIDS. Fat accumulation in the liver or muscle of any infant dying unexpectedly should suggest strongly the possibility of this or a related disorder of fatty acid oxidation.

**Very long-chain fatty acyl CoA dehydrogenase deficiency** is associated with similar clinical findings, although there also may be evidence of a cardiomyopathy. Infants with this and several other fatty acid oxidation defects may present with cardiac arrhythmias or unexplained cardiac arrest.

The accumulation of fatty acyl CoAs in patients with fatty acid oxidation defects leads to a secondary carnitine deficiency, probably as a result of excretion of excess acylcarnitines in the urine.

**Investigations**: Urine organic acid analysis, measurement of serum carnitine, and analysis of the plasma acylcarnitine profile are the most helpful laboratory studies in the initial screening for defects in fatty acid oxidation. These studies are sufficient to establish the diagnosis of medium chain acyl CoA dehydrogenase deficiency, which is associated with the presence of a characteristic metabolite, octanoylcarnitine, on the acylcarnitine profile. Since the organic acid abnormality often disappears when the child is apparently healthy, diagnosis may be difficult if urine and blood samples are not saved from the time when the patient was acutely ill.

**Enzymatic assays** may be necessary for the definitive diagnosis of some of the fatty acid oxidation defects.

**Treatment**: As is true for the defects in carbohydrate metabolism leading to hypoglycemia, treatment of the fatty acid oxidation defects involves avoidance of fasting and provision of adequate glucose. Restriction of dietary fat intake and supplemental L-carnitine therapy are recommended.

**C. Others**

When ketoacidosis is present at the time of hypoglycemia organic acidurias (methylmalonic, propionic, isovaleric, hydroxybutyric, methylglutaconic and methylcrotonic), ketolytic defects (succinyl co transferase, 3 hetothiolase), late onset Maple syrup urine disease (MSUD), and glycerol kinase deficiency should be considered.

In nutshell, approach to the etiology of hypoglycemia is based on 4 major clinical criteria (Fig.1) and few lab investigations. (Table 1)

- Liver size
- Characteristic of hypoglycemia (unpredictable, permanent, postprandial or only after fasting)
- Association with lactic acidosis
• Association with hyperketosis or hypoketosis

An algorithmic approach may also be helpful (Fig. 1)

Table 1. Key laboratory investigations during symptomatic hypoglycaemia

<table>
<thead>
<tr>
<th>Basic</th>
<th>Essential/Diagnostic</th>
</tr>
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<tbody>
<tr>
<td>• Blood gases</td>
<td>• Acyl carnitines (dried blood spots)</td>
</tr>
<tr>
<td>* Blood count</td>
<td>* Urine organic acid</td>
</tr>
<tr>
<td>* CRP</td>
<td>* Plasma aminoacids</td>
</tr>
<tr>
<td>* Electrolytes</td>
<td>* Serum/Plasma Free fatty acids and plasma ketones (3-Hydroxybutyrate)</td>
</tr>
<tr>
<td>* Phosphate</td>
<td>* Serum Hormones - Insulin, cortisol, growth hormone</td>
</tr>
<tr>
<td>* Liver/Renal function tests</td>
<td></td>
</tr>
<tr>
<td>* Creatine kinase</td>
<td></td>
</tr>
<tr>
<td>* Uric acid</td>
<td></td>
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<tr>
<td>* Triglycerides</td>
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</table>

Fig. 1. Approach to a child with IEM presenting with hypoglycaemia
Points to Remember

- **Diagnosis of IEM requires a high index of clinical suspicion.**
- **Presence of persistent vomiting, acidosis and seizures with normal sepsis screen points towards an IEM.**
- **The triad of hypoglycemia, marked hepatomegaly and lactic acidosis is characteristic of many gluconeogenesis defects.**
- **A stepwise correct choice of investigations can lead to a specific diagnosis and early management.**

References


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DDAVP has no effect on the sleep architecture of children with primary monosymptomatic nocturnal enuresis (PME) when analysed by classical, polysomnography which is determined by collecting the electric activity of cortical neurons. Taking recent research findings into account, this supports the thesis that the disturbances causing PME occur at brain stem level and do not reach consciousness.

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**Chiappini E, Conti C, Galli L, Maurizio de Martino. Clinical efficacy and tolerability of linezolid in pediatric patients: A systematic review. Clinical Therapeutics 2010;32: 66-88.**

The reviewed pediatric studies in skin and skin–structure infections, bacteremia or pneumonia found that linezolid was associated with high clinical cure rates that did not differ significantly from those of vancomycin or cefadroxil. RCTs enrolling children with other types of infection, as well as long–term studies are needed to draw definitive conclusions about linezolid’s efficacy and tolerability in pediatric patients. Careful monitoring for adverse events and possible linezolid resistance continues to be essential.
INBORN ERRORS OF METABOLISM PRESENTING AS HYPERAMMONEMIA IN NEONATES

* Lakshmi V
** Shanmugasundaram R

Abstract: Neonatal hyperammonemia is a medical emergency requiring prompt recognition and aggressive therapy. It is apparent that the clinical signs of hyperammonemia are non-specific and could be attributable to many serious illnesses of the neonate like sepsis, intraventricular hemorrhage, etc. Hyperammonemia can be primary or secondary. Urea cycle disorders and other inborn errors of metabolism though individually uncommon represent an important cause of hyperammonemia in neonates. Therapy is aimed towards minimizing endogenous production and removal of ammonia.

Keywords: Hyperammonemia, Neonates, Inborn errors of metabolism

Ammonia is a major waste product of nitrogen metabolism in all cells. In neonates, as nitrogen turnover is far greater than adults a larger amount of ammonia is produced and excreted. Ammonia is toxic to the nervous system and is excreted in the nontoxic form by going through the urea cycle. A significant exogenous source of ammonia comes from decomposition of urea and other nitrogenous compounds in the intestine by microorganisms. Primary hyperammonemia occurs due to urea cycle defects (UCD) and secondary in other conditions. There is a direct relationship between plasma ammonia levels and neurological status in patients with UCD and so is the neurological outcome.

Incidence

True incidence in general population is not known as many die even before diagnosis. The incidence is 1/30,000 births.\(^1\)

Normal plasma ammonium concentration in term and preterm babies vary according to the lab and method from 50-150µg/dL. Values greater than 75 µg/dL in term and 150µg/dL in preterm babies are high and need to be evaluated. Prompt chilling of heparinized blood and immediate transport to the laboratory is required to avoid false high levels.

Serum ammonia is measured by any of the four methods: 1. ammonium sensitive electrode, 2. enzymatic method, 3. cation exchange method and 4. alkaline diffusion method.

Aetiology

Conditions resulting in neonatal hyperammonemia and the various differential features are shown in Tables 1 and 2 respectively.

1. Urea cycle defects (UCD): Complete absence of any of the 5 enzymes results in primary hyperammonemia (Fig.1). With the exception of arginase deficiency all the other urea cycle defects present in the neonatal period. There is no metabolic acidosis but there is respiratory...
Table 1. Conditions resulting in neonatal hyperammonemia

<table>
<thead>
<tr>
<th>Urea cycle defects</th>
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<tbody>
<tr>
<td>Carbamyl phosphate synthetase deficiency</td>
</tr>
<tr>
<td>Ornithine transcarbamylase deficiency</td>
</tr>
<tr>
<td>Arginosuccinate synthetase deficiency (citrullinemia)</td>
</tr>
<tr>
<td>Argino succinate lyase deficiency</td>
</tr>
<tr>
<td>Transient hyperammonemia of prematurity</td>
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<table>
<thead>
<tr>
<th>Disorders of branched-chain amino acid metabolism</th>
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<tbody>
<tr>
<td>Beta–ketothiolase deficiency</td>
</tr>
<tr>
<td>Isovaleric acidemia</td>
</tr>
<tr>
<td>Methylmalonic acidemia</td>
</tr>
<tr>
<td>Propionic acidemia</td>
</tr>
<tr>
<td>Severe perinatal asphyxia</td>
</tr>
<tr>
<td>Total parenteral nutrition</td>
</tr>
<tr>
<td>Liver failure</td>
</tr>
<tr>
<td>Rare miscellaneous</td>
</tr>
<tr>
<td>Congenital lysine intolerance</td>
</tr>
<tr>
<td>Syndrome of hyperornithinemia-hyperammonemia-homocitrullinuria</td>
</tr>
<tr>
<td>Lysinuric protein intolerance</td>
</tr>
<tr>
<td>Syndrome of Rett (hyperammonemia-cerebral atrophy)</td>
</tr>
<tr>
<td>Hyperlysinemia with lysine induced crisis</td>
</tr>
</tbody>
</table>

The suspicion of organic acidemias. The most marked elevations occur in methylmalonic acidemia and propionic acidemia. The proposed mechanisms are accumulated propionyl coA which inhibits NAG synthesis and CPS requires saturating amount of NAG also and acetyl coA is required for NAG synthesis.

2. Organic acidemia and congenital lactic acidosis

Untoward accumulation of organic acid inhibits ureagenesis and varying degrees of hyperammonemia have been associated with organic acidemia though less severe than the primary form. Presence of wide anion gap metabolic acidosis, ketosis, hypoglycinemia, neutropenia and thrombocytopenia should arouse the suspicion of organic acidemias. The proposed mechanisms are accumulated propionyl coA which inhibits NAG synthesis and CPS requires saturating amount of NAG also and acetyl coA is required for NAG synthesis.

3. Transient hyperammonemia of newborn (THAN)

It is a self-limiting condition seen in stressed preterm neonates and is usually apparent in the first 12 hours of life. Serum ammonia levels have
Fig. 1. Steps of urea cycle

Fig. 2. Algorithmic approach to diagnosis of UCD
exceeded 1000μmol/L. Pathogenesis is still unclear though immature urea cycle has been proposed. Prognosis is good with early treatment and prompt reduction of ammonia level.

4. Other causes like perinatal asphyxia, liver cell failure, TPN etc.

Pathophysiology of central nervous system injury

Elevated levels of ammonia is toxic to the nervous system. It causes neuronal injury which is irreversible when severe and prolonged by the following mechanisms:

1. Interferes with energy metabolism in reticular activating system (RAS).

2. Disturbance of neurotransmitter metabolism and favours release of dopamine and norepinephrine from nerve endings.

3. Glutamine is a byproduct of ammonia metabolism which favours incorporation of tryptophan into the central nervous system which is a precursor for neurotonins, serotonin and quinolinic acid

4. Ammonia as a cation has a direct inhibitory effect on nerve impulse conduction.

Table 2. Differential diagnosis of neonatal hyperammonemia
ensues. Further elevation results in respiratory arrest, fixed-dilated pupils and sustained levels above 500μmol/L often cause increased intracranial pressure which may be irreversible.

**Inheritance**

Inheritance is autosomal recessive with the exception of ornithine transcarbamylase deficiency which is x-linked, wherein females are carriers and males are affected with disease. Family history for consanguinity and previous sibling neonatal deaths should be elicited.

Fig. 3. Approach to management in hyperammonemia
Investigations

1. Elevated serum ammonia levels (> 500 μmol/L)
2. Elevated glutamine and alanine (both are derived from ammonia)
3. Decreased citrulline (CPS or OTC defects)
4. Decreased arginine and urea levels
5. Elevated urinary orotic acids in all except CPS deficiency (carbamylphosphate when not utilized for urea synthesis results in formation of orotic acid in cytosol)
6. Urinary aminoacids and plasma aminoacids for citrullinemia and arginosuccinicaciduria
7. Serum electrolytes and pH (wide anion gap metabolic acidosis)
8. Blood gas analysis (respiratory alkalosis)
9. Liver function tests
10. Complete blood counts
11. BUN, glucose, lactate, pyruvate
12. Urine analysis – reducing substance, ketones, aminoacids

Principles of management

Apart from correcting shock, metabolic acidosis, dyselectrolytemia, hypoxia, infection and maintaining normoglycemia, therapy is aimed towards removal of ammonia and its immediate precursors glutamine, glutamate and alanine, by a combination of renal excretion of hippurate, citrulline and arginosuccinic acid (ASA) and peritoneal dialysis and hemodialysis.

1. Reducing the endogenous production of ammonia by proteolysis by restricting protein intake to 1.5g/kg/day, substitution of essential aminoacids and intravenous administration of 10-15% glucose.

2. Stimulation of alternate metabolic pathway of nitrogen excretion

   Administration of sodium benzoate, which is conjugated in the liver with glycine to form hippurate.

   Phenyl acetate conjugated in the liver with glutamine to form phenylacetylglutamine.

   Though phenyl acetate promotes more efficient removal of ammonia, it is not widely used in view of its offensive odour. These products are not recommended in secondary hyperammonemia like organic acidemia as they may worsen the acidosis.

3. Arginine supplementation

   Arginine should be supplemented as they are insufficient in UCD and are needed for normal growth. Arginine promotes citrulline and arginosuccinic acid production in citrullinemia and arginosuccinic aciduria which is excreted in urine and replaces urea as a major nitrogen carrier.

Drugs and dosages

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route</th>
<th>Dosage</th>
</tr>
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<tbody>
<tr>
<td>Arginine hydrochloride</td>
<td>IV / PO</td>
<td>0.2-0.8g/kg</td>
</tr>
<tr>
<td>Sodium benzoate</td>
<td>IV / PO</td>
<td>0.25-0.5g/kg</td>
</tr>
<tr>
<td>Sodium phenylacetate</td>
<td></td>
<td>0.25g/kg</td>
</tr>
<tr>
<td>Essential aminoacids</td>
<td></td>
<td>0.7g/kg</td>
</tr>
<tr>
<td>Pyridoxine*</td>
<td></td>
<td>5mg/day</td>
</tr>
<tr>
<td>Folic acid *</td>
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<td>0.1mg</td>
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*Co-factors for glycine synthesis

Megavitamins in congenital lactic acidosis and organic acidemia

Caloric intake 100-120 kcal/kg (Mead Johnson product 80056)
4. Removal of ammonium

Exchange transfusion: Is a readily available procedure and should be the first line procedure to lower serum ammonia levels. Fresh whole blood is preferred as older blood may contribute to elevated ammonia levels. Two volume exchanges are recommended although larger upto four volumes may be necessary in some babies. Repeated exchanges to as many as 10 exchanges have been necessary in some. It rapidly removes ammonia from the circulation. But it is not an ongoing procedure as dialysis.

Peritoneal dialysis: Although exchange transfusion has been recommended, recent studies show that peritoneal dialysis is more effective in removing the accumulated ammonium and improving the survival. Peritoneal dialysis is performed using a standard dialysis fluid containing 1.5% added glucose. Ammonia is easily dialyzable. Short 30 minutes cycles are used.

Hemodialysis: Hemodialysis may even be more effective because access to the circulation may be easily obtained by umbilical artery and venous catheterization. Small 30ml extracorporeal dialyzers are available. Continuous venovenous diafiltration was also done in the initial management of hyperammonemia.

Dialysis is continued for 36-60 hours once serum ammonia levels have come down baby will start breathing and the sensorium will improve. By this time the cause of hyperammonemia should be evaluated and appropriate therapy instituted.

GI drugs: Ammonia is also produced from intestinal bacteria hence administration of oral unabsorbed antibiotic like neomycin has been useful. Lactulose oral or rectal which acidifies the gut and promotes poorly absorbable ammonium has been tried. But above measures have questionable efficacy in acutely ill neonates.

Prognosis

The neurological and developmental outcomes appear to be related to the magnitude of hyperammonemia, duration of coma and rate of neurologic improvement. Although some acute neonatal hyperammonemic syndromes due to UCD were highly fatal with early aggressive therapy, Batshaw, et al reported nearly 50% of survivors with normal development and 25% with mild retardation.

Prenatal diagnosis

Prenatal diagnosis is possible in AS and AL deficiency because these enzymes are expressed in amniocytes. Further more ASA may be detected in the amniotic fluid. In OTC deficiency where sex determination is done and if male, pregnancy termination may be offered. In CPS deficiency sex determination is not useful.

Points to Remember

- Hyperammonemia incidence is under estimated in view of undiagnosed deaths.
- High index of suspicion in any acutely ill neonate especially if there is history of consanguinity and sibling death.
- Prompt diagnosis and early aggressive treatment may improve the neurological outcome in survivors.
- Neonatal diagnosis and general counseling should be offered to the couples.
- Hyperammonemia of newborn has a good prognosis if treated early and aggressively.
- Treatment modalities to remove ammonia from the circulation like PD, HD are necessary during acute episodes.
**References**


5. Lemley KV, Hintz RS, Enns GM. Continuous renal replacement therapy in the initial management of Neonatal Hyperammonemia Due to urea cycle defects. NeoReviews 2000;1.


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This study compared intramuscular and subcutaneous administration of two doses of measles–mumps–rubella–varicella (MMRV) combination vaccine (Priorix-Tetra™, GlaxoSmithKline Biologicals) in children. Healthy children (N = 328) were randomised to receive MMRV either intramuscularly or subcutaneously. Reactogenicity was similar between treatment groups for immediate vaccination pain, vaccination site pain, redness and incidence of fever and rashes. Slightly less vaccination site swelling occurred during days 0–3 of the post-vaccination period after intramuscular administration. Seroconversion rates for all components, 42–56 days post-dose 2, ranged from 99.3% to 100% in the intramuscular group and from 98.6% to 100% in the subcutaneous. Cell-mediated immunity data supported the humoral immunogenicity findings. In summary, the MMRV vaccine is well tolerated and highly immunogenic when administered either subcutaneously or intramuscularly to children in the second year of life.

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**Federico R. Laham, MD, Amanda A. Trott, MD, Berkeley L. Bennett, MD, MS, Claudia A. Kozinetz. LDH concentration in Nasal-Wash Fluid as a Biochemical Predictor of Bronchiolitis Severity. Pediatrics 2010; 125: 225-233.**

NW LDH levels in young children with bronchiolitis varied according to viral etiology and disease severity. Values in the upper quartile were associated with ~80% risk reduction in hospitalization, likely reflecting a robust antiviral response. NW LDH may be a useful biomarker to assist the clinician in the decision to hospitalize a child with bronchiolitis.
FATTY ACID OXIDATION DISORDERS

* Thangavelu S

Abstract: Fatty Acid Oxidation (FAO) disorder is an important group of inborn errors of metabolism. Clinical features develop during periods of fasting, because fatty acids are the major source of fuel. When diagnosed and treated early, they can be managed with simple dietary advice and can live a full life. If the physician is not aware of the features of FAO disorders, this may be mistaken as Reye syndrome or sadly parents may be blamed with the diagnosis of Munchausen Syndrome by Proxy. Any child with recurrent hypoglycaemia without ketones or with unexplained neuromuscular disorders or cardiomyopathy needs to be evaluated for FAO disorders.

Keywords: Fatty acid oxidation, SIDS, Hypoketotic hypoglycemia, Reye like syndrome, Cardiomyopathy.

Fatty acid oxidation disorder is an autosomal recessive inherited condition due to defects in the enzyme system that metabolizes the fatty acid (FA) by oxidation. Main source of energy for human body is glucose. During fasting, when glucose levels are low, alternate source of energy is ketone body, a metabolic product of FA, which comes from the β oxidation of fatty acids. Long chain FAs are a major source of energy for resting skeletal muscle and myocardium. Hence FAO disorders seriously affect the functioning of highly energy dependent tissues like heart, muscle, brain and liver. Children with Fatty Acid Oxidation disorders (FAO) do not get alternate energy when the serum glucose levels are low. When diagnosed and treated early, these children carry a good prognosis.

Pathophysiology

During fasting FAs are released into circulation from depot stores. They are taken up and oxidised for energy by most tissues except central nervous system. Because of this there will be sparing of glucose which will be utilized by CNS. Long chain FAs are transported into mitochondria by carnitine and other three transporter protein CPT I (Carnitine Palmitoyl Transferase), CPT II and Translocase. Once inside mitochondria, they pass through series of steps by enzymes of beta oxidation. Based on their chain length specificity, enzymes of beta oxidation are categorized as follows:

VLCAD: Very Long Chain Acyl CoA Dehydrogenase

LCAD: Long Chain Acyl CoA Dehydrogenase

MCAD: Medium Chain Acyl CoA Dehydrogenase

SCAD: Short Chain Acyl CoA Dehydrogenase

At the end of the β oxidation acyl coA is converted into acetyl coA, which is the substrate for energy production in Tricarboxylic Acid (TCA) cycle and for production of ketone bodies. Hence fatty Acid Oxidation (FAO) leads to defect in energy production and accumulation of acyl coA derivatives. Oxidation of FA in mitochondria is one of the major source of energy providing up to 80% of the requirement while fasting.
FAO disorders constitute a group of diseases caused by deficiency of transporter proteins such as carnitine, CPT I, Translocase or CPT II and enzymes involved in beta oxidation which are mentioned above.

MCAD deficiency is the most common one among the FAO disorders. A common point mutation is 985 A→G of the MCAD cDNA. Second most common disorder is LCAD deficiency and the common mutation is 1528 G→C.

**Clinical features**

There are four groups of clinical presentation because of defects in the FA oxidation.

1. Clinical features pertaining to skeletal and cardiac muscles because their main source of energy is from fatty acid, resulting in hypotonia, weakness, cardiomyopathy and cardiac failure.

2. Reye like episodes of hypoketotic hypoglycemia, hyperammonemia, hepatic failure and encephalopathy, which can occur because of fasting associated with illness. A metabolic crisis may be precipitated by simple illnesses like upper respiratory infection or otitis. Indicators of possible crisis are vomiting, diarrhea, excessive sleepiness, seizure and coma. If not properly diagnosed or suspected child may die without diagnosis or sometimes parents may be blamed with the diagnosis of Munchausen’s syndrome by proxy.

3. FAO disorders are considered as important cause of Sudden Infant Death Syndrome (SIDS) though there is no substantiation from molecular studies.

4. Very rarely mothers of affected fetus may develop acute fatty liver of pregnancy or HELLP syndrome (Hemolysis, Elevated Liver enzymes and Low Platelets)

**Diagnosis**

Evaluation of FAO disorders includes clinical suspicion and estimation of plasma free and total carnitine, glucose, ketone bodies, lactate, pyruvate and ammonia. Blood acyl carnitine analysis by TMS, urinary organic acids, measurement of enzymes and mutational analysis are other investigations. Following are the commonly seen lab findings that lead to the diagnosis.

- Hypoglycemia with inappropriately low or absent ketones.
- The first line diagnostic test is the analysis of acyl carnitine esters and its diagnostic metabolites.
- MCAD deficiency is characterized by elevated octanoyl carnitine
- Organic acid analyses and serum carnitine levels may be useful
- Increased urinary levels of mono and/or dicarboxylic acids, glycin conjugates and acylated carnitines. This reflects the intracellular accumulation of acyl-coA derivatives.
- Enzyme assay in fibroblasts and molecular analysis to confirm the mutation can be done in specialized laboratories.

**Treatment**

Objective of management of FAO disorder is to ensure that fatty acids are not used as major metabolic fuel (Table 1).

- Prevention of hypoglycemia is important by avoiding fasting for more than 8-12 hours and providing frequent low fat and carbohydrate rich meals at bed time. Vigorous treatment of respiratory infection and gastro-enteritis will prevent precipitating situations. Acute management includes intravenous glucose at the rate of 7-10 mg/kg/min with or without adding
insulin to maintain serum glucose around 100 mg/dl. One should remember the fact that excess glucose may increase lactic acidosis. Carnitine supplement is useful in carnitine deficiency at the dose of 100 mg/kg/day. It should be avoided in disorders with LCAD deficiency. Intravenous lipids are to be avoided but medium chain triglycerides (MCT) can be given in proven disorders of LCAD deficiency. In an acute situation, dialysis and exchange transfusion are found to be useful in disorders of Long Chain FA oxidation and the carnitine cycle. Riboflavin can be tried in the dose of 150 mg/day in ETF deficiency or SCAD deficiency. Potential use of fibrates to increase mutant protein levels and DL-3 Hydroxy Butyrate are the new concepts in the treatment.

**Table 1. Prevention and treatment of FAO disorders**

- Prevention of prolonged fasting.
- Early and vigorous treatment of infection.
- IV glucose infusion 7-10mg/kg/minute.
- Carnitine supplement 100mg/kg/day (except in LCHAD deficiency).
- Medium chain triglycerides
- Dialysis and exchange transfusion
- Riboflavin at 150mg/day.

**Prognosis**

When diagnosed and treated at birth, outcome in FAO disorders particularly MCAD deficiency is excellent, but more guarded in other disorders. But most can adjust to dietary advice during fasting and illness and can lead a full life.

**Points to Remember**

- *Fatty acid oxidation disorders are caused by deficiency of the transport protein or β oxidation enzyme system.*

- **Clinical presentation includes hypoketotic hypoglycemia, Reye like syndrome, involvement of skeletal and cardiac muscle.**

- **Rarely in mothers of affected fetus it can cause acute fatty liver of pregnancy.**

- **Management mainly involves avoiding fasting and providing frequent low fat and carbohydrate rich meals at bed time.**

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3. www.fodsupport.org/fods_defined.htm


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**Mitochondrial DNA and Diabetes Mellitus**

*Biswajit Mohanty
**Balasubramanian J*

**Abstract:** Diabetes mellitus affects approximately 5% of the general population with its prevalence varying between ethnic groups and geographic regions. The majority of cases are either type 1 or type 2 diabetes. Although these disorders share a common phenotype, fasting and postprandial hyperglycemia, their etiology is distinct. A growing body of evidence has demonstrated a link between mitochondrial functioning and type 2 diabetes. Certain mitochondrial DNA (mtDNA) mutations affect insulin secretion involving an attenuation of ADP/ATP levels leading to a re-setting of the glucose sensor in the pancreatic β-cell. Co-morbid conditions include impaired hearing, changes in pigmentation of the retina, gastrointestinal abnormalities, cardiomyopathy, and MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes).

**Keywords:** Type 2 Diabetes, Mitochondrial DNA, Comorbid Conditions.

Diabetes mellitus can be defined as a state of chronic hyperglycaemia sufficient to cause long-term damage to specific tissues, notably the retina, kidney, nerves and arteries. Maintenance of normal glucose homeostasis involves the action of a glucose sensor in the pancreatic β-cell that detects an increase in blood glucose concentration and converts that into increased secretion of insulin. The ability of pancreatic β-cell to sense ambient glucose levels accurately and rapidly depends on the glucose transporter isoform GLUT-2, enzyme glucokinase and the pulsating ratio of ATP/ADP. The ATP helps in expelling insulin present in the secretory vesicles into the extracellular space (exocytosis), from where insulin and C peptide enter the islet capillaries. The synthesis of ATP takes place in the inner membrane of mitochondria on respiratory chain by a process called oxidative phosphorylation. The mutation of mtDNA has been observed to lead on to diabetes.¹ ²

**Mitochondrial DNA**

Human mtDNA is a double helical circle containing 16,569 base pair. A striking feature of the human mitochondrial genome is its extreme economy. Nearly every base pair in human mtDNA encodes a protein or a RNA product. This genome encodes 13 proteins, 22 tRNAs, 2 rRNAs, NADH reductase, proton-pumping complexes, cytochrome reductase and oxidase subunits and ATP synthase subunits i.e. components responsible for oxidative phosphorylation or ATP generation in the cell.

mtDNA is vulnerable to mutation compared to nuclear DNA because it is composed almost exclusively of coding sequences, lacks protection by histones, has inefficient repair mechanisms. It is also exposed to reactive oxygen species (ROS) produced during oxidative phosphorylation while generating ATP.³ ⁴ ⁵

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Molecular defect

Mutation of mtDNA occurs by substituting guanine for adenine (A-to-G) at position 3243 of leucine transfer RNA (tRNALeu(UUR)).\(^6\) Interestingly, tRNALeu(UUR) is a hot spot for mutations; 10 disease-related mutations have been identified within this gene, 4 of which are associated with diabetes mellitus. Nearly all the carriers developed diabetes or IGT before the age of 70 years; thus, the penetrance of this mutation is nearly 100%. Diabetes can be type 1 or type 2 in nature depending on the severity of insulinopenia.

Mitochondrial function in beta cell insulin secretion: Type 2 diabetic patients have an impaired ability of the pancreatic beta-cells to secrete insulin.\(^7\) Glucose-stimulated insulin secretion (GSIS) has been characterized by its pulsatile nature as generated by oscillations in the ATP/ADP ratio.\(^8\) A series of steps must be completed inside and outside the mitochondria before insulin can be secreted. This increase in ATP/ADP ratio causes the ATP-sensitive K\(^+\) channels to close, causing depolarization of voltage-sensitive Ca\(^{2+}\) channels.\(^9,10\) The depolarization triggers the opening of specific (voltage-gated) Ca\(^{2+}\) channels in the membrane. Ca\(^{2+}\) ions then flood into the β cell from the outside and activate the contractile proteins which drag the secretory vesicles containing insulin and C peptide to the cell surface. Here, the vesicles fuse with the cell membrane and release their contents into the extracellular space (exocytosis), from where insulin and C peptide enter the islet capillaries. The processing of proinsulin to insulin within the secretory granules of the Golgi complex is dependent on critical levels of pH (3.5-7.4) as well as ATP.\(^11\)

Mitochondrial inheritance

Mitochondrial inheritance behaves differently from autosomal and sex-linked inheritance. Nuclear DNA has two copies per cell (except for sperm and egg cells). One copy is inherited from the father and the other from the mother. Mitochondria, however, contain their own DNA, and contain typically from five to ten copies, all inherited from the mother. Mutations to mtDNA occur frequently, due to the lack of the error checking capability that nuclear DNA has. This means that mitochondrial disorders occur spontaneously and relatively often. Mitochondrial disease begins to become apparent once the number of affected mitochondria reach a certain level; this phenomenon is called “threshold expression”.

Clinical presentation

This disorder is associated with diabetes mellitus and deafness. It is inherited maternally (MIDD). A variety of studies have determined that of patients with type 1 and type 2 diabetes mellitus, the A/G exchange is present in 0.5–1.5% of patients. If only those patients with a family history of diabetes are considered, the prevalence of the mutation is two to five times higher. In a cross sectional study in Netherlands among diabetes patients the prevalence of MIDD was reported to be 1.3%.\(^12\) The prevalence of the mitochondrial mutation in a Japanese study was 4.6% in diabetic patients with maternal inheritance and/or hearing loss.\(^13\) Similar finding were reported in a Chinese study.\(^14\) There is a paucity of Indian data on MIDD.

Characterization of affected individuals and their siblings demonstrated that 48% presented with diabetes and deafness, 21% had diabetes alone, 15% had deafness alone, 3% had deafness associated with some neurologic changes, and 13% had diabetes, deafness, and findings of MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes).
Although patients with mtDNA mutations were initially diagnosed as having either type 1 or type 2 diabetes mellitus, there were differences between these patients and those with type 1 or type 2 diabetes. Ketosis has been described rarely. Pancreatic antibodies are generally not a feature of MIDD, although islet-cell antibodies have been described. Diabetes that is not insulin-requiring at outset can usually be managed with dietary treatment or oral hypoglycemic agents, although there is fairly rapid conversion to insulin dependance.

Those initially diagnosed as having type 2 diabetes tend to be leaner and more likely to be treated with insulin compared with general populations with type 2 diabetes. Subsequent studies to define the etiology of diabetes in patients with mitochondrial mutations demonstrated decreased insulin secretion in response to an oral glucose tolerance test, whereas measures of glucose utilization demonstrated essentially normal insulin sensitivity. The above findings suggest that the primary defect in patients with mitochondrial mutations is in the pancreatic β cell, oxidative phosphorylation and ATP generation.

Management

In patients with diabetes or IGT accompanied by hearing loss and maternal history of diabetes, a diagnosis of mtDNA mutations as the cause of the diabetes should be considered. Family members need to be carefully screened for both diabetes and evidence of sensorineural hearing. Frequently, the hearing loss develops after the onset of diabetes, so those patients with diabetes need to be examined over time for hearing loss. The data suggests that patients who replace two percent of the energy from trans-fatty acids (TFA) with polyunsaturated fatty acids could decrease the risk for type 2 diabetes by as much as 40 percent. TFA contributes to mtDNA mutation. So patients should be advised to avoid food containing TFA. Patients with these mutations are likely to require insulin therapy because of the insulin deficiency that develops sooner or latter. Metformin group of drugs should be avoided as it may lead to lactic acidosis and further aggravate the situation.

Conclusion

Type 2 diabetes is not merely a disease of insulin insensitivity or lack of insulin release but may be a global dysfunction of the mitochondrial energy system. The expression of this phenotype seems dependent on a critical threshold associated with mutations. Although reduction of mtDNA is a critical factor in type 2 diabetes pathology, the question as to the nature of the original insult, dysregulated ROS or some external protoxins or environmental chemicals remains to be answered.

Points to Remember

• Type 2 diabetes mellitus may not be mere insulin insensitivity or release; it may be part of a global dysfunction of mitochondrial energy system.

• Mutation of mtDNA as a cause is to be considered in diabetes with hearing loss and maternal diabetes.

• Patients with mutation require insulin in due course.

• Metformin group of drugs are to be avoided as they lead to lactic acidosis.

References


CLIPPINGS


No severe adverse effects were observed. Mild sedation was reported in one infant. All six patients treated with oral LEV became seizure free within 6 days. Five patients remained seizure free after 3 months with ongoing LEV monotherapy. One infant developed pharmacoresistant epilepsy. Seizures relapsed later in the clinical course of two more patients, one of whom was no longer under LEV therapy. Results from this small patient group indicate that LEV may be an alternative therapeutic option in neonatal seizures.
APPROACH TO ANASARCA

* Maiya PP
** Sharanabasavesh M

Abstract: Generalized edema otherwise known as anasarca, is a common presentation of various conditions. Common causes include renal, cardiovascular, nutritional and hepatic diseases. Majority of children presenting with anasarca may have diagnosis referable to any of the systems mentioned. Occasionally in a child with anasarca there may be difficulty in diagnosis for which a systematic approach will help. The approach should include carefully taken history, clinical examination and basic investigations. An occasional child may require extensive investigation for the diagnosis.

Keywords: Anasarca, Edema, Hypoalbuminemia, Child.

Anasarca refers to generalized edema characterized by puffiness of face, most noticeable in the periorbital area and by persistence of indentation over the medial aspect of ankle following pressure.1 Majority of children with anasarca have straight forward etiology related to cardiovascular, nutritional, renal or hematological condition. Occasionally in some such children there may be problem in ascertaining the etiology. Hence a systematic approach will be necessary to diagnose the cause of generalized edema.

Table 1 lists the causes for anasarca, which will help to arrive at a diagnosis in a child with anasarca.

Table 1. Causes of anasarca

<table>
<thead>
<tr>
<th>Cardiac</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCF secondary to congenital heart disease, cardiomyopathy, myocarditis, arrhythmias, rheumatic heart disease, hypertension, cor pulmonale, severe anemia, thyrotoxicosis.</td>
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</table>

<table>
<thead>
<tr>
<th>Renal</th>
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<tr>
<td>Nephrotic syndrome, glomerulonephritis, renal failure.</td>
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</table>

<table>
<thead>
<tr>
<th>Nutritional</th>
</tr>
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<td>Kwashiorkor</td>
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<table>
<thead>
<tr>
<th>Hepatic</th>
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<tbody>
<tr>
<td>Fulminant hepatic failure, autoimmune hepatitis</td>
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<tr>
<td>Drug induced liver failure: Acetaminophen, Isoniazid, Phenytoin</td>
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<tr>
<td>Metabolic diseases causing liver failure: Wilson disease, glycogen storage disease type IV, mitochondrial disorders, Alpha-1, antitrypsin deficiency, Niemann-Pick disease.</td>
</tr>
<tr>
<td>Hypo perfusion of liver: Budd Chiari syndrome, Veno-occlusive disease.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Gastro intestinal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory bowel disease, gluten sensitive enteropaty, cystic fibrosis, intestinal lymphangiectasia, trypsinogen or enterokinase deficiency, milk protein sensitivity, eosinophilic gastroenteropathy, short bowel syndrome.</td>
</tr>
</tbody>
</table>
Approach to anasarca

Approach to any particular disease necessitates a proper history and physical examination. This helps in limiting the differential diagnosis to specific organ system in a child with anasarca.

History

**Cardiac:** Children with anasarca due to cardiac disease present with the complaints of feeding difficulties, excessive sweating, failure to thrive, respiratory distress and cyanosis. Older children may also have history of orthopnea, dyspnea and syncopal symptoms. Edema usually occurs in the dependent area i.e. sacral area in infants and ankle region in toddlers and older children.

**Liver:** Ask for history of fever, anorexia, vomiting, pain abdomen, progressive jaundice, fetor hepaticus, bleeding manifestation and abdominal distension. In patients with liver disorder edema usually starts as ascites and progresses to develop generalized edema. Infants may initially present with irritability, lethargy, poor feeding, and sleep disturbance.

**Nutritional:** This is the commonest cause of anasarca in most of the third world countries. Properly taken dietetic history, socioeconomic history, and growth parameters help in making the diagnosis of nutritional cause for anasarca. Early in the disease symptoms include anorexia, lethargy, apathy and irritability. Later they present with failure to thrive, increased susceptibility to infection and edema.

**Renal:** Children with renal disorder present with decreased urine output, hematuria and edema. Edema is in the form of early morning facial puffiness, which gradually decreases as the day progresses in the initial stage of the disease, which later on becomes generalized and persistent. History of repeated urinary tract infection, history of sore throat or skin infection in the recent past and symptoms related to hypertension should be asked for.

In addition history should include history of chronic diarrhoea, progressive pallor, drug intake, insect bite and burns.

Clinical examination

General Examination should include looking for peripheral signs of liver cell failure, congestive cardiac failure, rheumatic heart disease and anemia. One has to also look for manifestations of vitamin and mineral deficiencies.

**CVS:** Heart failure occurs when cardiac output is inadequate to meet the metabolic needs. On examination along with edema other features of congestive cardiac failure like tachycardia, tachypnoea, basal rales, tender hepatomegaly and cardiomegaly are looked for. Features of poor peripheral circulation like cold extremities prolonged capillary filling time and low blood pressure can be noticed. Edema is seen in the dependent areas initially which becomes generalized. Most common causes of CCF in young infants and children are due to congenital heart defects E.g., VSD, PDA, hypoplastic left heart syndrome, TGA, etc. Cardiomyopathy which occurs due to number of causes could be a cause for CCF. Other causes include viral myocarditis, severe anemia, hyperthyroidism, AV malformation, etc.

**Liver disease:** On examination of a child with liver disease and anasarca icterus may also be a feature. Increase in liver size initially, which gradually shrinks without clinical improvement may be seen in chronic liver disease. Edema starts as ascites which later on becomes generalized as the disease progress. Mental status changes are noted with progression of disease. Signs of liver cell failure like, fetor hepaticus, spider nevus, asterixis, palmar erythema, testicular atrophy, etc develop as the disease progresses.
Renal: Edema occurring in acute phase of glomerulonephritis is characteristically associated with hematuria, hypertension and oliguria. The edema in this condition is primarily due to retention of sodium and water by the kidneys. On examination some times pyoderma may be noticed. The age group is generally between 5-15 yrs of age.

Edema in nephrotic syndrome is primarily due to massive urinary loss of protein leading onto hypoalbuminemia. Most commonly it is seen in children between the ages of 2 and 6 years. Episodes may be precipitated by minor infections. They usually present with mild edema around the eyes and in lower limb that decreases over the day. As the disease progresses edema becomes generalized. The child may have associated oliguria, anorexia, irritability, abdominal pain and diarrhoea.

Nutritional and Gastrointestinal: Kwashiorkor is a classical example of nutritional deficiency state leading to anasarca. It is characterized by growth retardation, psychomotor changes and edema. Edema starts in the lower extremities and later involves upper limb and the face. Associated features like hair changes, skin changes, hepatomegaly, recurrent infections, vitamin and mineral deficiency signs help in making the diagnosis easier.

Gastrointestinal losses of protein either due to chronic diarrhoea, digestive enzyme deficiency, protein losing enteropathy, inflammatory bowel disease, cystic fibrosis, etc lead to anasarca.

Investigations

With proper history and physical examination differential diagnosis for anasarca can be narrowed down and appropriate investigation can be asked for. Hence basic investigations like hemogram, urine examination, renal function test, liver function test, chest x-ray may suggest the cause for anasarca. When the basic investigations do not confirm the diagnosis, further investigation may have to be done. One such investigation would be ascitic fluid analysis that helps in differentiating whether ascites is secondary to portal hypertension or due to non-portal hypertension causes.

Ascitic fluid analysis: In the past, ascites was classified on the basis of protein content either as exudative (more than 2.5g/dL) or transudative (less than 2.5 g/dL). It had its own drawbacks with high false positive results. Presently this has been replaced by serum, ascites albumin gradient (SAAG) that is calculated by subtracting ascitic fluid albumin from serum albumin. It is classified as portal hypertensive (SAAG > 1.1g/dL) or high albumin gradient ascites which was previously known as transudative ascites and non portal hypertensive (SAAG < 1.1g/dL) or low albumin gradient ascites which was previously known as exudative ascites. The accuracy of the SAAG results is approximately 97% in classifying portal hypertensive and non-portal hypertensive ascites. A high gradient is associated with diffuse parenchymal liver disease and occlusive portal and hepatic venous disease.

Table 2 summarizes the causes for ascites based on SAAG values.

Management

The management depends upon the etiology. Child with renal disease may require fluid restriction and diuretics, while child with CCF may require bed rest, digoxin, diuretics and vasodilators. Child with hepatic cause may have gross hypoalbuminemia which requires dietary supplementation, salt free albumin etc. Occasionally one may come across a child with undiagnosed anasarca. These are the patients who may require extensive investigations to rule out the rarer causes like intestinal lymphangiectasia.
and cystic fibrosis where relevant investigations like sweat chloride test, endoscopy may have to be carried out.\textsuperscript{10}

Flowchart (Fig.1) summarizes the approach to the diagnosis of anasarca in the child.

**Points to Remember**

- *Edema more in the morning and subsiding by evening is suggestive of renal edema.*
- *Ascites to start with, followed by edema may suggest a possibility of hepatic failure.*
- *Nutritional history combined with anthropometry, vitamin and mineral deficiency signs, points to the diagnosis of nutrition deficiency states like kwashiorkor.*
- *Edema in the dependent part associated with tachypnoea and abnormal findings in the heart suggest the diagnosis of cardiovascular conditions for anasarca.*

### Table 2. Causes of ascites based on SAAG\textsuperscript{8}

<table>
<thead>
<tr>
<th>High gradient ascites (SAAG &gt; 1.1 gm)</th>
<th>Low gradient ascities (SAAG &lt;1.1 gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhosis</td>
<td>Nephrotic syndrome</td>
</tr>
<tr>
<td>Venoocclusive disease,</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>Fulminant hepatic failure</td>
<td>Nutritional</td>
</tr>
<tr>
<td>Cardiac ascites</td>
<td>Collagen vascular disorder</td>
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<tr>
<td>Mixed ascites</td>
<td></td>
</tr>
<tr>
<td>Liver metastasis</td>
<td></td>
</tr>
<tr>
<td>High SAAG , normal protein</td>
<td>Budd chiari syndrome, constrictive pericarditis</td>
</tr>
<tr>
<td>High SAAG , low protein</td>
<td>Cirrhosis</td>
</tr>
<tr>
<td>Low SAAG , low protein</td>
<td>Nephrotic syndrome, tuberculosis, nutritional</td>
</tr>
<tr>
<td>Low SAAG , normal protein</td>
<td>Chylous ascites, pancreatic ascites</td>
</tr>
</tbody>
</table>

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Viral bronchiolitis is a leading cause of acute illness and hospitalization of young children. Research into the variation in treatment and outcomes for bronchiolitis across different settings has led to evidence–based clinical practice guidelines. Ongoing investigation continues to expand this body of evidence. Authors of recent surveillance studies have defined the presence of coinfections with multiple viruses in some cases of bronchiolitis. Underlying comorbidities and young age remain the most important predictors for severe bronchiolitis. Pulse oximetry plays an important role in driving use of health care resources. Evidence–based reviews have suggested a limited role for diagnostic laboratory or radiographic tests in typical cases of bronchiolitis. Several large, recent trials have revealed a lack of efficacy for routine use of either bronchodilators or corticosteroids for treatment of bronchiolitis. Preliminary evidence suggests a potential future role for a combination of these therapies and other novel treatments such as nebulized hypertonic saline.

CONTRIBUTOR TO CORPUS FUND OF IJPP

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Rs.500/-
MACROLIDES IN CHILDREN

* Jeeson C Unni

Abstract: Macrolides are useful and safe antibiotics for pediatric infections, especially if the child is allergic to penicillin and/or cephalosporin. They can be used to treat streptococcal pharyngitis, other respiratory tract infections, community-acquired pneumonia and cutaneous infections.

Keywords: Macrolides, Erythromycin, Azithromycin, Clarithromycin, Antibacterial spectrum, Indications, Dosage, Pharmacokinetics, Drug interactions, Adverse effects.

Macrolides are useful in treating gram positive infections especially when the child is allergic to penicillins and cephalosporins. Currently available macrolides in common use in India are erythromycin and the newer macrolides clarithromycin and azithromycin. Erythromycin, though effective, has a number of disadvantages, including a narrow anti-bacterial spectrum, unfavorable pharmacokinetic properties such as a short serum half life, and frequent GI intolerance. Increasing resistance of group A streptococci to erythromycin has been reported in the West. Though isolated studies in India show a trend towards this phenomenon, others, including the ongoing prospective hospital surveillance of invasive pneumococcal disease in seven referral hospitals in India, under India CLEN network report very low resistance to erythromycin. Resistance appears to correlate with the amount of macrolide use within a community as evidenced by a decrease in erythromycin resistance among group A streptococci associated with a nationwide decrease in macrolide use in Finland.

The newer macrolides, clarithromycin and azithromycin, are synthesized by altering the erythromycin base to produce compounds that possess longer half-life allowing once or twice daily administration, resistance to gastric degradation, improved oral bioavailability and possibly better GI tolerance, excellent tissue and intracellular penetration and an extended spectrum of activity. They can therefore be administered for children who do not tolerate erythromycin or have infections with organisms resistant to erythromycin and as an alternative to co-amoxiclav or second or third generation cephalosporins.

Mechanism of action, antibacterial spectrum and clinical uses

Macrolide antibiotics act by binding to the P site of the 50S ribosomal subunit of susceptible organisms and by inhibiting bacterial RNA-dependent protein synthesis. They may be either bacteriostatic or bactericidal, depending on the drug concentration and bacterial susceptibility. Although generally bacteriostatic, clarithromycin and azithromycin are bactericidal against Streptococcus pyogenes, Streptococcus pneumoniae and Haemophilus influenzae. These agents are also useful in managing pertussis and infections caused by Legionella,
Chlamydia, and Mycoplasma.\textsuperscript{7,8} Clarithromycin is several-fold more active in vitro than erythromycin against gram-positive organisms.\textsuperscript{9} Azithromycin in vitro is considered less potent than erythromycin against gram-positive organisms but this may not be clinically significant as susceptibility concentrations fall within the range of blood levels attainable with azithromycin. Further, azithromycin appears to be more active than erythromycin against many Gram-negatives and several other pathogens, notably Haemophilus influenzae, H. parainfluenzae, Moraxella catarrhalis, Neisseria gonorrhoeae, Urea plasma urealyticum and Borrelia burgdorferi.\textsuperscript{9,10} Azithromycin has excellent in vitro activity against H influenzae, whereas clarithromycin, although less active against H influenzae in vitro, is metabolized into an active compound, 14-hydroxyclarithromycin, with twice the in vitro activity of the parent drug. Oral azithromycin and clarithromycin are equally effective against respiratory tract and soft tissue infections by S aureus, S pneumoniae, S pyogenes, H influenzae, and M catarrhalis that are susceptible to these drugs. Thus, these 2 drugs are rational choices for the treatment of community-acquired pneumonia. However, the low serum concentrations of azithromycin may be a problem in patients with bacteremia associated with community-acquired pneumonia.\textsuperscript{11} Clarithromycin appears more active than azithromycin and erythromycin against Legionella pneumophila and Chlamydia pneumonias, whereas azithromycin demonstrates better in vitro activity against M. catarrhalis and Mycoplasma pneumonias.\textsuperscript{12,13} Clarithromycin appears to be effective for the treatment and prophylaxis of Mycobacterium avium complex (MAC) in patients with AIDS, while azithromycin appears to be effective for prophylaxis.\textsuperscript{11}

Though azithromycin is the only macrolide with extended gram negative cover, its only significant clinical use is its effectiveness against Salmonella typhi.\textsuperscript{14} This Cochrane review suggests that azithromycin appears better than fluoroquinolone drugs in populations that included participants with drug-resistant strains and that it may perform better than ceftriaxone. Clarithromycin is the macrolide of choice in triple regimens for the treatment of Helicobacter pylori.\textsuperscript{15}

Parenteral azithromycin has been used as an alternate treatment for toxoplasma encephalitis in AIDS patients.\textsuperscript{16} The newer macrolides are also prescribed for the prophylaxis and therapy for disseminated Mycobacterium avium complex disease in patients infected with the human immunodeficiency virus.\textsuperscript{17}

Topical erythromycin is used in treatment of acne vulgaris. Oral azithromycin and erythromycin have been used for the treatment for acne vulgaris in adolescents but there are recent reports of resistance to both oral and topical erythromycin.

The common uses in day-to-day practice are as follows:

**Erythromycin**
- Alternative to penicillin in penicillin allergy for secondary prophylaxis in rheumatic fever
- Mild to moderate infections of skin
- Atypical pneumonia
- Neonatal conjunctivitis due to C trachomatis
- Eradication of B pertussis from nasopharynx/prophylaxis

**Clarithromycin**
- Eradication regimens for H.pylori
- Atypical pneumonia

Cost and bad taste are disadvantages.
Azithromycin

- Atypical pneumonia – drug of choice
- Short course therapy for AOM > 2 yr age
- Skin infections

Newer macrolides are preferred to erythromycin in acute otitis media, sinusitis and pneumonia due to higher activity against H. influenzae.

Pharmacokinetics in children

The bioavailability of oral macrolide antibiotics is poor. At best, only 40% to 50% of a given dose is absorbed. Food significantly reduces the absorption of azithromycin as capsules and the stearate and some base forms of erythromycin. Therefore, they need to be taken at least 1 hour before or 2 hours after food. Clarithromycin; azithromycin suspension; and erythromycin in the estolate, ethylsuccinate, and delayed-release base (enteric-coated) forms can be taken without regard to meals. In fact, when taken with meals, the oral bioavailability of clarithromycin is increased by up to 25%.

The macrolides penetrate well into the respiratory, genitourinary and GI tracts as well as into skin, soft tissues, and sinuses. They are only moderately (40% to 50%) protein-bound. Erythromycin and clarithromycin are mainly metabolized by the liver. The metabolites of erythromycin are excreted in the bile and urine to a small extent. Clarithromycin is metabolized to a pharmacologically active compound, 14-hydroxy clarithromycin, which undergoes renal elimination. None of the macrolides cross the blood brain barrier.

Although serum concentrations of azithromycin are low following oral administration, high tissue levels are attained; achieving tissue half-life of approximately three days. This enables the clinician to use once daily dosing and ensures that a five-day single daily dose regimen for respiratory tract and soft tissue infections will provide therapeutic tissue concentrations for at least ten days. It also allows a single 1 g dose of azithromycin for the effective treatment of C. trachomatis genital infections. Clarithromycin has a longer serum half-life and better tissue penetration than erythromycin, allowing twice-a-day dosing for most common infections.

Drug interactions

Both erythromycin and clarithromycin inhibit the activity of the hepatic cytochrome P-450 enzyme system. As a result, these agents reduce the metabolism and increase the serum concentration of other drugs eliminated via the P-450 pathway. Azithromycin, because of differences in its chemical structure, does not cause these interactions. Thus, theophylline clearance may be decreased by up to 35% when clarithromycin or erythromycin are prescribed concurrently; causing clinically important interactions especially when theophylline serum concentrations are already in the high therapeutic range (i.e., > 15 mcg/ml). Clarithromycin and erythromycin may inhibit the metabolism of cyclosporine via inhibition of the CYP3A4 isoenzyme, thus increasing cyclosporine’s effects and the potential for toxicity. It has been recommended to avoid cyclosporine in combination with these macrolide agents or reduce the cyclosporine dosage by 50% when it is necessary to give any of these macrolides concurrently.

The other medications known to be affected by erythromycin or clarithromycin are anticoagulants, carbamazepine, cisapride, digoxin and methylprednisolone. Erythromycin, clarithromycin, and azithromycin have been associated with the development of ventricular
arrhythmias, including torsades de pointes and ventricular tachycardia, in patients with a prolonged QTc interval.\textsuperscript{21} In many of the reported cases, these patients were also receiving a medication that interacts with a macrolide.

**Adverse events**

Most adverse reactions associated with the use of macrolide antibiotics are mild and resolve on discontinuing therapy. Because of the low incidence of toxic effects reported during the more than 55 years of their use, macrolides are considered to be the safest antimicrobial agents available,\textsuperscript{22} so a review of their adverse effects deals mostly with rare and unusual events.

In children, the most frequently reported adverse effects with erythromycin are diarrhea, vomiting (6% each), abdominal pain, rash (3% each), nausea (1% to 2%), and headache (2%). Less frequent but more severe adverse effects include anaphylaxis, Stevens-Johnson syndrome, reversible hearing loss, and hepatocellular cholestatic hepatitis.\textsuperscript{23} Pseudomembranous colitis, resulting from the overgrowth of Clostridium difficile, has long been associated with erythromycin.

Azithromycin and clarithromycin appear to be well-tolerated. Gastrointestinal intolerance is the primary adverse side effect of both antimicrobials but occurs at a significantly reduced rate when compared to erythromycin.\textsuperscript{18} Azithromycin causes diarrhea [3.6%], nausea [2.6%], abdominal pain [2.5%], and headache or dizziness [1.3%]. Only 0.7% of patients discontinued azithromycin therapy as compared to 2.6% of patients receiving comparative medications.\textsuperscript{24} The most common adverse reactions of clarithromycin are nausea [3.8%], diarrhea [3.0%], abdominal pain [1.9%], and headache [1.7%].\textsuperscript{1} Laboratory abnormalities were also rare and included elevated liver function tests and decreased white blood cell counts. Overall, fewer than 3% of patients receiving clarithromycin withdrew from studies because of adverse effects.\textsuperscript{25} Though adverse effects have been mild, there have been isolated reports of pseudomembranous colitis (long been associated with erythromycin) in patients on clarithromycin.\textsuperscript{26}

Nephrotoxicity caused by macrolides must be considered a rare adverse event. Very few data are available in published case reports, either for adults or children, that show strong evidence for a causal relationship.\textsuperscript{27,28} No evidence of nephrotoxicity from clarithromycin has however been found in clinical trials.

**Dosages and administration**

**Erythromycin**

Dosage: General indications: Neonates – Orally/IV < 7 days age 20mg.kg/day 2 times daily; > 7 days <1200gm 20mg.kg/day 2 times daily; > 7 days >1200gm 30mg.kg/day 3-4 times daily

Children: Orally - 30-50 mg/kg/day in 4 divided doses max 250mg 4 times daily may be given. 12-18yrs – 250-500mg 4 times daily. IV – 1mth – 18yr 12.5mg/kg/dose 4 times daily or as a continuous infusion. Maximum dose - 4 g/day. Replace by oral dosage as soon as possible.

Special indications : Topical (acne vulgaris): Wash and apply twice daily directly to the affected area.

Secondary prevention of rheumatic fever – when child is sensitive to penicillin - oral 20mg/kg/day max 500mg twice daily (Contraindicated in liver disorder)

Chlamydia trachomatis pneumonia in infants and neonates: Oral - 50 mg/kg/day (erythromycin base) in four divided doses for 14 days.
Ophthalmia neonatorum caused by Chlamydia trachomatis: Neonates – Oral - 50 mg/kg/day in four divided doses for 14 days. If chlamydial conjunctivitis recurs after discontinuing therapy, the erythromycin dosage regimen should be repeated.

A beta-hemolytic streptococcal (GAS) pharyngitis (primary rheumatic fever prophylaxis as an alternative to penicillin in children allergic to penicillin)- 40 mg/kg/day in 4 divided doses x 10 days. Cardiology sub chapter of IAP does not recommend using erythromycin for this indication.

Uncomplicated urethral, endocervical, or rectal gonorrhea, penicillinase-producing Neisseria gonorrhoeae, or for gonorrhea during pregnancy - Adolescents: 500 mg PO four times per day for 7 days

Treatment and postexposure pertussis prophylaxis – For postexposure prophylaxis, administer to close contacts within 3 weeks of exposure, especially in high-risk patients (e.g., women in 3rd trimester, infants < 12 months). Oral dosage: Infants, Children, and Adolescents: 40-50 mg/kg/day PO (maximum 2 g/day) in four divided doses for 14 days. For neonates: Azithromycin is preferred. If azithromycin is unavailable, erythromycin 40-50 mg/kg/day PO in 4 divided doses may be used. Monitor for infantile hypertrophic pyloric stenosis.

Pneumococcal prophylaxis - 1 month - 2yr 250mg/day, 2-8 yr 500 mg/day, >9 yr 1gm/day in 2 divided doses.

Gastric stasis – Oral/IV – 1 month – 18yr - 3mg/kg 4 times daily.

Administration: Oral: total daily dose may be given in 2 divided doses, but gastrointestinal side-effects can occur.

Patients with hepatic impairment: Use with caution in patients with impaired hepatic function. Although specific dosage guidelines are not available, a reduced dosage may be necessary.

Patients with renal impairment: No dosage adjustment needed.

Azithromycin

Dosage: For all indications: 6 months - 12 years 10mg/kg/day once daily for 3 days up to maximum of 200 mg (3-7 years), 300 mg (8-11 years, 400 mg (12-14 years) and 500 mg > 14 years. Alternatively, 10mg/kg/day once on first day followed by 5mg/kg/day once daily from day 2 – day 5.

Group A beta-hemolytic streptococcal (GAS) pharyngitis and tonsillitis (primary prophylaxis of rheumatic fever): 12.5 mg/kg/day - single dose for 5 days. (Not recommended for secondary prophylaxis of rheumatic fever)

Chlamydial infection such as non-gonococcal urethritis (NGU) or cervicitis due to susceptible strains of Chlamydia trachomatis: Adolescent: single dose of 1 g orally.

Bacterial endocarditis prophylaxis: 15 mg/kg (single dose max 500mg) 30-60 minutes before procedure in children and adolescents allergic to penicillin.

Treatment and postexposure pertussis prophylaxis – (For postexposure prophylaxis, administer to close contacts within 3 weeks of exposure, especially in high-risk patients (e.g., women in 3rd trimester, infants < 12 months). Oral dosage: Infants > 6 months and Children: 10 mg/kg/day (maximum 500 mg) on day 1, then 5 mg/kg/day (maximum 250 mg) on days 2—5. Infants < 6 months: 10 mg/kg/day for 5 days. Monitor for infantile hypertrophic pyloric stenosis in infants < 1 month old.
Uncomplicated typhoid fever: Orally -
Adolescent: 8—10 mg/kg/day once daily for 7 days (1000 mg on first day, followed by 500 mg once daily for 6 days). Children: 10 mg/kg/day once daily for 7 days or 5-day regimen of 20 mg/kg/day.

Administration: Oral; administer 1 hour before or 2 hours after food. If a child vomits within 5 minutes of the dose, an additional dose may be given. If child vomits between 5-60 minutes of the dose, alternative therapy should be considered. If a child with normal gastric emptying, vomits 60 minutes or thereafter, no additional dose is warranted.

Clarithromycin

Dosage: Oral 15mg/kg/24hr in 2 divided doses up to maximum of 500mg twice daily for 5-10 days.

H. Pylori - 1-2 yr 125mg, 2-6yr 250mg, 6-9yr 375mg, 9-12yr 500mg and 12-18yr 1gm/day in 2 divided doses along with amoxycillin and omeprazole or amoxicillin and lansoprazole or metronidazole and omeprazole.

CAP and pharyngitis in children due to Chlamydomphila pneumoniae, Mycoplasma pneumoniae or Streptococcus pneumoniae give for 10 days.

Bacterial endocarditis prophylaxis: 15 mg/kg (single dose max 500mg) 30-60 minutes before procedure in children and adolescents allergic to penicillin.

Treatment and postexposure pertussis prophylaxis – (For postexposure prophylaxis, administer to close contacts within 3 weeks of exposure, especially in high-risk patients (e.g., women in 3rd trimester, infants < 12 months). Oral dosage: Infants > 6 months and Children: 15 mg/kg/day up to maximum of 500mg twice daily for 7 days. Not used in neonates.

Patients with renal impairment: Creatinine clearance (CrCl) > 60 ml/min: no dosage adjustment needed. CrCl 30-60 ml/min: No dosage adjustment needed except in patients receiving concurrent ritonavir. In these patients, reduce the recommended clarithromycin dose by 50%.

CrCl < 30 ml/min: reduce recommended dose by 50%. In patients receiving ritonavir, decrease the recommended clarithromycin dose by 75%.

Administration: Oral; food does not affect extent of bioavailability but may delay onset of absorption.

Conclusion

The use of macrolides in pediatric therapeutics in India is much less than that of beta lactam antibiotics. But it has its place in childhood infections and it must be used wherever it is recommended as a first line drug. Since it is not recommended for use in bacteremic illnesses these drugs are used mainly in OPD practice. The macrolide class as a whole includes some of the safest anti-infective agents available and therefore they must be used more often when indicated.

Points to Remember

- **Macrolides are the safest group of antimicrobial drugs available.**

- **Erythromycin is a good alternative to penicillin in penicillin allergy in mild to moderate infections by susceptible organisms. Also useful in treating atypical pneumonia and neonatal C.trachomatis conjunctivitis. It eradicates B. pertussis**
from the nasopharynx (reduces period of infectivity but does not alter course of disease). GI side effects is a drawback.

• Clarithromycin is used in eradication regimens for H. pylori. Cost and palatability are drawbacks.

• Azithromycin is highly concentrated in tissues allowing short course therapy with once daily dosing.

• Newer macrolides are preferred to erythromycin in otitis media, sinusitis and non bacteremic pneumonia due to better H. influenza and M catarrhalis cover and atypical mycobacteria infections.

References


Pyuria was not always sterile in patients with Kawasaki Disease (KD). Although there was no different clinical phenotype or coronary outcome in KD patients with or without UTI, the authors suggest that UTI should be considered and evaluated in KD patients with pyuria, a positive nitrite test or a positive result of urine culture. If UTI is definitively diagnosed, the patient should be treated for a UTI as well as for KD and complete post–UTI work–up is recommended.
DRUG ERUPTIONS – AN OVERVIEW

*Anandan V

Abstract: Adverse drug reaction is a common problem in clinical practice posing high degree of confusion in diagnosis and management. It may lead to fatal results if there is a delay in the initiation of the appropriate management. A proper knowledge about the various morphological patterns of drug eruptions and proper management will reduce the morbidity and mortality.

Keywords: Adverse drug reaction, AGEP, SJS, TEN.

In spite of limited information on the incidence of adverse drug reactions (ADR) in children, a meta analysis of prospective studies revealed an overall incidence of 9.5% of ADR among hospitalised children and the rate of pediatric hospital admissions due to ADR was 2.1% of which 39.3% were considered to be life threatening reactions. The incidence of ADR in outpatient setting was reported as 1.5%.

Cutaneous eruptions are one of the most frequent presentations of ADR and are found to be present in 40% of the ADR patients which range from mild erythematous rashes to life threatening toxic epidermal necrolysis (TEN) which could prove fatal.

Approach

The first and foremost in a patient with suspected ADR is early diagnosis and appropriate management which can reduce the mortality and morbidity.

Methodical and precise approach towards the morphological reaction pattern of ADR will give the treating physician a clue about the probable drug, as different drugs are commonly associated with specific types of reaction, such as exanthematous, urticarial, blistering or pustular and among these exanthematous and urticarial with or without angio oedema are the common types of eruptions. The reactions could be simple or complex with the presence of fever and systemic manifestations in the latter.

Drug exposure

ADR could be due to single or multiple drugs and it is essential to inquire about the concomitant usage of herbal and naturopathic remedies.

Most of the drug reactions occur within 1-6 weeks after the drug exposure and sometimes may take even 3 years as in the case of drug induced lupus.

Morphological approach

1. Exanthematous

Exanthematous drug rash is the commonest form of ADR accounting nearly 95% of the cases of which maculopapular is the commonest and the others being morbilliform and scarletiniform. The reaction can occur from 1-10 days of the
therapy and pruritis is the most common associated symptom. Resolution occurs within 7-14 days ending with scaling and desquamation.

The common drugs which are implicated for this type of reactions include penicillin, sulfanomides, barbiturates and anti epileptics. An exanthematous rash could be life threatening if it occurs with fever when one should suspect drug hypersensitivity syndrome (DHS) which is a triad of fever, skin eruptions and internal organ involvement. DHS can occur even with the first exposure of the drug starting from 1-6 weeks of drug exposure and these rashes have predilection towards the previous radiotherapy sites.

Even though fever and malaise could be the first symptoms, pharyngitis and cervical lymphadenopathy also occur early. The initial signs of eruption are edema and swelling of the face followed by erythema and pruritis of the face and subsequent caudal spread which can easily slip into Steven Johnson syndrome (SJS) or TEN. Internal organ involvement results in hepatitis, pneumonitis, pancreatitis, vasculitis, encephalitis, aplastic anemia, SIADH, myositis and thyroiditis.

The drugs associated with this type of reactions are aromatic anticonvulsants like phenytoin, carbamazepine, phenobarbitol and others like lamotrigine, sulphanomides, antibiotics, dapsone, minocycline and allopurinol.4

2. Urticarial

Urticarial reactions are characterized by the appearance of erythema and wheals with or without pruritis which may last from 1-48 hours and sometimes even for 5-6 days. These reactions could be IgE mediated (penicillins and antimicrobials) or non IgE mediated (narcotic analgesics, radio contrast media, acetyl salicylic acid and NSAIDS).

Latex allergy is a type 1 reaction which can range from simple urticaia to death, especially in children with spina bifida where they are exposed to catheters and latex products more frequently.5

3. Serum sickness like reaction (SSLR)

SSLR is defined by fever, rash (exanthematous or urticarial), arthralgia occurring 1-3 weeks after drug exposure which could be associated with lymphadenopathy and eosinophilia; however absence of immune complexes, hypocomplementemia, vasculitis and renal lesions differentiates SSLR from true serum sickness.

Epidemiological evidence in children suggests that the risk of SSLR is more with cefaclor than with other antibiotics including other cephalosporins.6 Recently cefprozil, minocycline, bupropion are reported to cause SSLR.

4. Pustular

a) Acneform eruptions: Acneform eruptions typically occur over the arms, legs, back and chest which are usually monomorphic papules and nodules without comedones sparing prepubertal children indicating that prior hormonal priming is a prerequisite.

The drugs that have been reported producing this type of reaction are steroids, iodides, bromides, ACTH, INH, androgens, lithium and actinomycin-D. This reaction can occur within 2 weeks of drug intake and is directly proportional to dosage and duration of the offending drug.7

b) Acute generalized exanthematous pustulosis (AGEP): Even though AGEP is rare in children, the estimated incidence is 1-5 cases/million/year.8

The reaction is characterized by an acute onset with a temperature more than 38°C and a
cutaneous eruption composed of sterile non follicular pustules on an erythematous and edematous base which may be associated with target lesions, vasculitis, blisters and erosions of the mucus membranes.

Resolution occurs by desquamation within 2 weeks after the withdrawal of the offending drug (β lactams, macrolides and calcium channel blockers); Leucocytosis is common and disappears before desquamation. Eosinophilia, hypocalcemia and renal failure have been reported with AGEP.

5. Bullous eruptions

a) Pseudo porphyria: A phototoxic disorder which is non dose dependent and has neither age nor sex predilection can resemble porphyria cutanea tarda (PCT) or erythropoietic protoporphyrina (EPP) in which normal porphyrin levels is indicative of pseudo porphyria. Clinically this can present with increased skin fragility, blistering, burning sensation and scarring over the sun exposed areas.

Pseudo porphyria has an estimated incidence of 12% in patients with juvenile rheumatoid arthritis receiving naproxen, oxaprozin and ketoprofen. Other drugs reported are furosemide, tetracycline and erythropoietin. Blistering may continue for weeks and skin fragility may persist for months. Resolution is slow and complete except for scarring.

6. Erythema multiforme major (EMM), SJS and TEN

Even though a strong debate is existing in distinguishing each of these entities by definition, it has been accepted that all these are different facets of the same disease process and arbitrarily it has been accepted that EMM is with 10%, SJS with 10-30% and TEN with more than 30% involvement.

Anticonvulsants, antibiotics (penicillins, sulfanomides), allopurinol and NSAIDS (piroxicam) are reported to produce the above said reactions.

The reaction may be acute or insidious with the onset of fever, malaise and anorexia which may last for 1-2 days. Patients with SJS may have the involvement of mucus membranes with erosions and hemorrhagic crusting ,blisters, ulceration with grey white membrane formation. Patients with TEN initially develop morbilliform or diffuse painful erythema rapidly resulting in blisters and peeling of skin in sheets with positive Nikolsky sign. (skin peeling with lateral pressure). Pseudo membrane formation over the cornea may lead to visual impairment.

Fluid and electrolyte disturbances and systemic complications do occur which have to be tackled cautiously.

7. Miscellaneous drug eruptions

a) Neutrophilic eccrine hidradenitis (NEH): In spite of its rarity, it has been reported to occur 11 days after starting the chemotherapy for acute myelogenous leukemia or lymphoma. The most common clinical presentation is edematous erythema, papules and plaques over face and trunk associated with neutropenia and fever. Resolution is complete and spontaneous with recurrences in which case dapsone helps in prevention of recurrences.

b) Fixed drug eruptions (FDE): FDE with incidence of 22 %, is clinically characterized by oval, pruritic circumscribed, erythematous, plaques occurring over the same sites with the re-challenge of offenders. The common sites involved are lips, trunk, legs, arms and genitals. Bullous FDE has been reported and the resolution occurs with hyper pigmentation.

The common offenders are sulphonamides, barbiturates, salicylates, acetaminophen, NSAIDS and tetracyclines.
c) Drug induced lupus (DIL): DIL is defined by the development of ANA with at least one clinical symptom of lupus erythematosus. The drugs reported for DIL are minocycline, procainamide and hydralazine.\textsuperscript{13} The eruption occurs after several weeks to 2 years which could be urticarial, erythematous or vasculitic with arthritis, hepatitis and with positive ANA.

\textbf{Differential diagnosis}

ADR should be mainly differentiated from acute viral exanthematous fevers (Drug or Bug), collagen vascular disease, Kawasaki disease and neoplasia which is possible with proper history, high index of suspicion and proper knowledge of the various morphological patterns of drug reactions produced by specific drugs.

\textbf{Management}

Even though the diagnosis of ADR is based on the drug history and clinical morphological patterns of reactions by specific drugs, investigations can also serve as corroborative evidence. CBC may reveal eosinophilia, neutropenia in a case of NEH and leucocytosis in AGEP. ANA will be positive in DIL. Oral provocation test and intra dermal skin test could be done to confirm the drug hypersensitivity and should be done with caution.

Patch testing will be helpful in FDE. Research oriented investigations like radio immune sorbent assay (RAST), lymphocyte transformation test and lymphocyte cytotoxicity assay could throw some light on better understanding of ADR. Skin biopsy will be helpful in TEN to differentiate it from Staphylococcal scalded skin syndrome where the split will be at the dermo epidermal junction in the former, where as in the latter it will be more superficial. Treatment is mainly avoiding the offenders. Early initiation of steroids either topical or systemic, anti histamines, nutrition, fluid and electrolyte management will reduce the morbidity and mortality. High dose intravenous gamma globulins (hdIVG) will be life saving in the case of TEN.

Even though the usage of steroids is controversial, in Indian scenario the usage of steroids is advocated. Topical steroids of mid potent strength is recommended for milder reactions and systemic steroids like prednisolone 1-2mg/kg/day or 3-4 weeks with gradual tapering, injectable dexamethasone 0.2mg/kg/day for 5-7 days is life saving. Currently 2.5-3g/kg/day for 3 days of intravenous gamma globulins are recommended to tackle severe reactions like TEN. Thalidomide was found to be detrimental in the management of TEN as it causes paradoxical enhancement of tumour necrosis factor alpha.\textsuperscript{14}

\textbf{Conclusion}

Adverse drug reaction can be properly managed by early diagnosis and appropriate management which will reduce the morbidity and mortality.

\textbf{Points to Remember}

- \textit{Early diagnosis and management is rewarding.}
- \textit{Steroids are life saving, especially in TEN.}
- \textit{Supportive measures are a must.}

\textbf{References}


Review of one small randomised controlled trial showed that honey was significantly better than no treatment for the relief of cough, reducing bothersome cough, improving child’s sleep; but no better than ‘no treatment’ in reducing the severity of cough and parent’s sleep. The effects of honey on symptom relief and sleep quality did not differ from those of dextromethorphan, which is a common ingredient in cough medications. Parents of five children assigned to honey and two assigned to dextromethorphan reported their children suffered from insomnia, hyperactivity and nervousness. However, as with other medications, its benefit should be considered alongside the adverse effects. This review has a limitation in that the results were obtained from a single study involving a relatively small number of children.
PHAKOMATOSIS

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Phakomatosis and vascular malformations also come under the group of disorders of cellular migration and neuronal organization. Phakomatoses refer to a group of neurocutaneous syndromes.

Neurofibromatosis is one of the more common types of phakomatosis that is well known because of its clinical features. Its hallmark feature is the neurofibroma, which is a tumor of the Schwann cells surrounding the peripheral nerves. There are two types of neurofibromatosis (NF). The first type is NF-1, which is the most common type and its clinical features include cafe au lait spots and neurofibromas. The radiological features in NF-1 include optic pathway gliomas, sphenoid wing dysplasia and thinning of the cortex of the long bones. The second type, NF-2, only accounts for 10% of the cases of neurofibromatosis, and its clinical feature is bilateral vestibular schwannomas or acoustic neuromas.

Tuberous sclerosis is another phakomatoses that presents with seizures early in life. These seizures are due to the presence of multiple hamartomas that are collections of dysplastic cells. They may be seen anywhere in the brain. But the characteristic finding in CT is the calcified subependymal nodule that is seen in the outer wall of the lateral ventricle (Fig.1). Another peculiar brain lesion in tuberous sclerosis is the giant cell astrocytoma. They are similar to the subependymal nodules but with a tendency to grow. They usually occur along the caudo-thalamic groove near the foramen of Munro and therefore present with hydrocephalus.

Renal involvement occurs in about 80% of patients with tuberous sclerosis. The lesions include angiomyolipomas, cysts and renal cell carcinomas. Angiomyolipomas are localized proliferations of blood vessels, smooth muscle

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Fig.1. Tuberous sclerosis-Subependymal calcific nodules
and fat. In ultrasound they appear as multiple, hyperechoic round lesions in the kidneys (Figs. 2a and 2b).

Sturge Weber syndrome consists of cutaneous vascular nevus and leptomeningeal angiomatosis. The angiomatosis causes hypoxia and atrophy of the subjacent brain which gives rise to the characteristic features seen in CT and MRI. Calcification with a tram track appearance conforming to the gyral pattern is seen in the cortex ipsilateral to the nevus. If sufficiently dense they can be seen in the plain xray of the skull and this is what was originally described by Weber. Location of the brain involvement is related to the particular area of the trigeminal nerve where the nevus is located. If the nevus is in the area of the ophthalmic division, the occipital lobe is affected. If the nevus is in the maxillary region, the parietal convexity is affected. The extent of angiomatoses and therefore the atrophy correlates to degree of seizure control and psychomotor development. The child in Fig. 3 also had congenital glaucoma, one of the ocular manifestations seen in Sturge-Weber syndrome. Note the calcified
undulating lines posteriorly on the right corresponding to calcified gyri. Compare the volume of the brain parenchyma on either side. The right hemisphere is atrophic and the subarachnoid spaces are dilated. Contrast CT shows a white blotch in the calcified portion corresponding to the angiomatosis (Fig.4). Calcification and contrast enhancement are both white in colour and cannot be separated from each other in CT. This disadvantage is overcome in MRI where the angiomatoses is brought out clearly with gadolinium enhancement. The enhanced vascular structures are white while calcification is black.

A few other phakomatosis are listed here for completion. Klippel-Trenaunay-Weber Syndrome consists of port-wine hemangiomas, osseous and soft tissue hypertrophy and deep parenchymal vascular malformations. Osler-Weber-Rendu disease or hereditary hemorrhagic telangiectasia presents in adolescence with multiple vascular malformations of the skin, mucosa, lung, brain, spinal cord, and other viscera. MRI is the imaging modality of choice to identify multiple cavernomas or capillary telangiectasias in the brain.

Ataxia telangiectasia consists of ocular and cutaneous telangiectasias and cerebellar atrophy with predominant involvement of the anterior vermis and dentate nuclei.

Though the above disorders are called neurocutaneous there is a large mesenchymal component in the form of vascular malformations and hamartomas. Apart from revealing the intracranial involvement, imaging also helps in defining this aspect.

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**NEWS AND NOTES**

**XXII NATIONAL CONFERENCE OF THE INDIAN SOCIETY OF PEDIATRIC NEPHROLOGY**

**Venue : Swabhumi, Kolkata, India**

**Dates :**
- Workshop on Renal Imaging : 12th November 2010
- Conference : 13th & 14th November 2010

**Organised by**

*Indian Society of Pediatric Nephrology*

*West Bengal Academy of Pediatrics*

**Conference Secretariat**

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CASE STUDY

INCONTINENTIA PIGMENTI WITH MACROCEPHALY

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Abstract: Incontinentia pigmenti (IP) is an X linked dominant disorder of skin which is often associated with ocular, dental and central nervous system abnormalities. Though life expectancy is normal, quality of life is dependent on the associated abnormalities.

Keywords: Incontinentia pigmenti.

Incontinentia pigmenti is a rare heritable, multi system disorder that is transmitted as an X linked dominant trait, lethal in males. Incontinentia pigmenti is characterized by skin lesion, microcephaly, seizure and dental abnormalities. The rarity of incontinentia pigmenti with macrocephaly that too in a male child was the reason for presenting the case

Case Report

A six-month-old male infant born to third degree consanguinous marriage was referred in view of generalized seizures. On examination, he was thriving well with a weight of 7 kgs. There was no anemia or lymphadenopathy. He had hyperpigmented whorly lesion involving trunk, back, both upper and lower limbs. There was no lesion over face. Central nervous system examination revealed macrocephaly (head circumference-47cms; 97th percentile) with microphthalmia, wide open anterior fontanelle with sunset sign. The tone in both upper and lower limbs was increased and the deep tendon reflexes were brisk. Fundus examination was suggestive of severe retinal pigmentary changes. Cardio vascular system and respiratory system were essentially within normal limits. There was no organomegaly. Investigations revealed normal total and differential counts. ESR, urine microscopy and metabolic screen were normal. Electrolytes and other metabolic parameters were normal. USG abdomen was normal. EEG showed abnormal epileptiform discharges. MRI of brain showed right hippocampal atrophy with dilated right temporal horn, small sized left hippocampus with patchy T2 hyper density with suspected left mesial temporal sclerosis. The infant was started on anticonvulsants. With the features of seizures, hyperpigmented whorly lesion of the skin, retinal pigmentary changes and macrocephaly a diagnosis of incontinentia pigmenti with macrocephaly was made.

Discussion

Incontinentia pigmenti (IP) is a rare X linked, dominantly inherited disorder of skin pigmentation that is often associated with ocular, dental and central nervous system abnormalities. Its incidence is 1 case in 40,000. It is more common in whites than in other races. IP is a lethal syndrome in males. More than 95% of reported cases occur in females. IP may rarely occur in males with Klinefeter syndrome (XXY) or as a result of somatic mosaicism or less deleterious gene mutation. The male to female ratio is 1:19-37. Gorrod described the first patient in 1906, Sulzberger described pathological
changes in 1928 and Haber first recognized the multi system nature of disease.\(^5\) Happel first recognized the skin changes along line of Blaschko in 1985. Incontinentia pigmenti is characterized by loss of melanin from basal cells in epidermis. Melanin collects in the dermis as aggregates of melanophages. It is caused by a genomic rearrangement of gene for NEMO, Nuclear factor kappa beta essential modulator.\(^6\) The defect in the X chromosome is proximal to factor 7 at Xq28. Two third of mutation originates with father. NEMO, the ubiquitous protein becomes active during embryogenesis and is essential for blood vessel architecture, cell growth in stratified epithelium and apoptosis.\(^7\) The skin manifestations occur along the line of Blaschko, which represent the route of embryonic cell migration. Diagnosis is made clinically on the basis of a history of sequential cutaneous lesions and associated features.\(^3\) At least one major criterion is necessary for diagnosis of sporadic incontinentia pigmenti (neonatal rash, hyperpigmentation, linear, atropic, hairless lesion.) At least one female relative who was previously diagnosed with incontinentia pigmenti may be diagnosed with minor criteria (dental involvement, wooly hair, abnormal nail, retinal disease). Skin change is present in 80% of cases and it involves mostly the sides of torso. Stage 1 is vesicular stage with linear vesicles, pustules and bullae with erythema along lines of Blaschko. Stage 2 is verrucous stage with warty, keratotic papules and plaque. Stage 3 is hyper pigmented stage with macular hyper pigmentation in a swirled pattern along the lines of Blaschko. It involves nipple, axilla and groin. Stage 4 is

![Fig. 1. Macrocephaly with sunset sign](image)

![Fig. 2. Hyperpigmented whorly lesions in back](image)
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Hypo pigmented streaks and patches and cutaneous atrophy. Alopecia and scarring occur in 40% of children. In one third of children developmental delay, seizures, microcephaly, spasticity and paralysis occurs. Ocular impairment and blindness is seen in 15% of children. Late dentition, conical teeth and partial anodontia are other components. Incontinentia pigmenti must be differentiated from conditions like microophthalmia, dermal aplasia and sclerocornea (MIDAS) syndrome, linear epidermal nevus, lichen striatus, linear and whorled nevoid hypermelanosis, dermatopathia pigmentosa reticularis. Hypomelanosis of Ito is characterised by mirror image lesions of IP, but with swirls of hypopigmentation or depigmentation. It is not inherited. There is no stage 1 or 2. There can be multisystem involvement (eye, skeletal, neurologic) in 33-50% of cases. It is associated with Xp11.

No specific treatment is available for incontinentia pigmenti. Stage 1 lesion should be left intact and kept clean. Regular consultations with ophthalmologist, dentist, geneticist and neurologist are advised. The skin abnormalities can improve with age and in some instances disappear completely. Prognosis in IP depends on the severity of extra cutaneous manifestations. Morbidity and mortality primarily result from neurologic and ophthalmic complications including mental retardation, seizures and vision loss. IP is a geno dermatosis and can be associated with malignancies such as acute myelogenous leukemia, Wilms tumour and retinoblastoma.

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Fig. 3. Typical hyperpigmented whorly lesions of trunk

hypo pigmented streaks and patches and cutaneous atrophy. Alopecia and scarring occur in 40% of children. In one third of children developmental delay, seizures, microcephaly, spasticity and paralysis occurs. Ocular impairment and blindness is seen in 15% of children. Late dentition, conical teeth and partial anodontia are other components. Incontinentia pigmenti must be differentiated from conditions like microophthalmia, dermal aplasia and sclerocornea (MIDAS) syndrome, linear epidermal nevus, lichen striatus, linear and whorled nevoid hypermelanosis, dermatopathia pigmentosa reticularis. Hypomelanosis of Ito is characterised by mirror image lesions of IP, but with swirls of hypopigmentation or depigmentation. It is not inherited. There is no stage 1 or 2. There can be multisystem involvement (eye, skeletal, neurologic) in 33-50% of cases. It is associated with Xp11.

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CONFERENCE - On 27th, 28th November 2010.

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