Mitochondrial Hepatopathies

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Abstract
Hepatocyte mitochondrion functions as a cause and as a target of liver injury. Since the mitochondria are under dual control of nuclear DNA and mitochondrial DNA (mtDNA), mutations in genes of both classes have been associated with inherited mitochondrial hepatopathies. Point mutations, deletions, insertions, rearrangements, DNA depletion—all have been identified. Many factors influence the prevalence of mitochondrial disorders, including the mutations rate, inheritance pattern, population structure, and the genetic background. In primary disorders, mitochondrial defect is the primary cause of liver disease often producing fatal hepatic failure in infancy or childhood. In secondary disorders, insult to mitochondria is caused by either a gene defect that affects non-mitochondrial proteins or by an exogenous injury to mitochondria. Diagnosis should be suspected in cases of liver disease with neuromuscular symptoms, multisystem involvement that cannot be explained by a single pathology or rapidly progressive liver failure in early childhood. Laboratory findings in the blood and urine show an altered redox status. Various anti-oxidants, vitamins, cofactors, and electron acceptors have been for proposed but none is effective. Presence of neuromuscular or extraintestinal involvement in primary disorder precludes the use of liver transplantation. ©

INTRODUCTION

Mitochondria are important cellular organelle responsible for energy production through oxidative phosphorylation (OXPHOS). Mitochondrial dysfunction yields deficient oxidative phosphorylation, increased generation of reactive oxygen species (ROS), and accumulation of hepatocyte lipid, impairment of other metabolic pathways and activation of both apoptotic and necrotic pathways of cellular death.1

Mitochondrial genome: Mitochondria are able to replicate, transcribe, and translate their DNA independently. The mitochondrial DNA (mtDNA) genome is a circular, double-stranded molecule that encodes for 2 ribosomal RNAs: 22 transfer RNAs, and 13 polypeptides of complexes I, III, IV, and V of the respiratory chain.2 The characteristic of mtDNA includes:2,3
(1) Maternal inheritance with variable expressivity of phenotype.
(2) Heteroplasmy (a mixture of normal and abnormal mitochondria in the affected cell)
(3) Random segregation with resultant shift in the clinical phenotype with age.
(4) High mutability due to high information density and lack of redundancy.

Mutations in genes of both nuclear DNA and mtDNA are associated with inherited mitochondrial disorders.2 An estimated 90% of mitochondrial diseases are caused by mutations in nuclear genes, with only a small fraction of these mutations having been identified at this time.2,3 As a general rule, point mutations of mtDNA genes are usually maternally inherited whereas deletions or rearrangements of mtDNA are either sporadic or inherited in an autosomal recessive manner (caused by mutations in nuclear genes).3

Mitochondrial proteins: The majority of the estimated 1000 different mitochondrial proteins are encoded by nuclear DNA, translated in the cytoplasm and transported into the mitochondria. The production of ATP occurs through respiratory chain, which is divided into five multienzyme complexes (proteins) embedded in the inner mitochondrial membrane.1 They are: (1) complex I: NADH-CoQ reductase, (2) complex II: succinate-CoQ reductase, (3) complex III: reduced CoQ-cytochrome c reductase, (4) complex IV: cytochrome c oxidase, and (5) complex V: ATP synthase.

Mitochondrial disorders: Mitochondrial disorders pose particular problems for the genetic researchers.2 The clinical presentation varies considerably and many patients present with non-specific features, leading to significant delay in diagnosis; moreover transmission of a pathogenic mutation does not always produce a
Mitochondrial disorders were once regarded as neuromuscular diseases, with most mutations found in mtDNA. However, symptoms involving any organ system have been reported, and a frequent feature is an increasing number of organs involved as the disease progresses over time. Thirty to 56% of patients with mitochondrial disorders have non-neuromuscular manifestation at presentation. Mitochondrial DNA deletions are notable for their hepatic presentations, with or without neuromuscular involvement, and typically result in death from liver failure.

**MITOCHONDRIAL HEPATOPATHIES**

Sokol and Treem have proposed a classification scheme for mitochondrial hepatopathies (Table 1). In primary disorders, mitochondrial defect is the primary cause of liver disease. Leonard and Schapira have divided them into those caused by mutations affecting mtDNA genes (class 1a) and those caused by mutations in nuclear genes that encode mitochondrial respiratory chain proteins or cofactors (class 1b). The secondary disorders, in which hepatic mitochondria undergo injury or dysfunction caused by another pathologic process, include diseases of uncertain etiology (e.g., Reye’s syndrome), exposure to endogenous and exogenous toxins, drugs (such as nucleoside analogues), or metals, and other conditions in which mitochondrial oxidative injury or abnormal electron transport may be involved (e.g., cholestasis, NASH).

**Review of selected diseases**

**Reye’s syndrome** - This best known form of (secondary) mitochondrial hepatopathy is caused by the interaction of viral infection (influenza, varicella, enterovirus, other viruses) and salicylate use with some underlying undefined metabolic (defects in fatty acid oxidation) or genetic (reduced activity of an uncoupling protein) predisposition. Salicylate impairs mitochondrial fatty acid oxidation by reversible inhibition of LCHAD activity. The affected child (usually between 5 to 15 years) appears to be recovering from a viral illness after 3 to 5 days when sudden, unremitting vomiting develops followed by encephalopathy. The liver dysfunction is characterized by elevated transaminases, with mild to moderate prolongation of prothrombin time, variable hypoglycemia but normal serum bilirubin. Liver biopsies show microvesicular steatosis in the absence of hepatic inflammation or necrosis and characteristic swelling and pleomorphism of mitochondria under electron microscopy. The liver makes a full recovery with supportive therapy.

**Wilson’s disease** - The underlying defect in Wilson’s disease is caused by mutations in the P type ATPase, ATPase7B, which is present in the trans-golgi and seems to be transported to hepatic mitochondria. Abnormal mitochondrial morphology characteristic of this disorder are: decreased matricreal density, enlarged intermembranous spaces, dilatation and vacuolization of cristae, crystalline inclusions, and vacuoles in the matrix. The accumulation of excess copper in hepatic mitochondria leads to oxidant stress with subsequent lipid peroxidation, and oxidative alterations of thiol-containing proteins. There may also be deletion of mitochondrial DNAs in young adults.

**Iron overload conditions** - In hereditary hemochromatosis and other conditions of iron overload,
iron-induced lipid peroxidation has been identified in membrane fractions from hepatocyte mitochondria, microsomes, and lysosomes. Membrane-dependent functions are often abnormal at iron concentration at which oxidative damage occurs. Kupffer cells phagocytose damaged iron-loaded cells resulting in their subsequent activation and production of fibrogenic compounds.

**Drugs and toxins -** Acquired abnormalities in mitochondrial respiration is caused by several drugs and toxins. The anticonvulsant Valproate is metabolized to 4-envalproic acid, a mitochondrial toxin and precipitate liver failure in those with underlying complex I or cytochrome c oxidase deficiency. Several toxins (cyanide, antimycin A, rotenone, cereulide) inhibit specific protein complexes of the respiratory chain. Experimental antiviral nucleoside, dialuridine (FIALU), when first used in trials in adults with chronic hepatitis B, was found to produce unexpected fatal lactic acidosis and liver failure due to its incorporation directly into mtDNA replacing thymidine. Other nucleoside analogues like zidovudine, didanosine, stavudine and zalcitabine have been shown to inhibit the DNA polymerase gamma and may block the elongation of mtDNA. Lamivudine, which is not incorporated into mtDNA, has not been associated with significant hepatic toxicity.

**Hydrophobic bile acid toxicity in cholestasis -** Hydrophobic bile acids and metals that accumulate in the liver during cholestasis may lead to perturbations of mitochondrial membrane function, hydroperoxide generation, resultant opening of the permeability pore, reduced OXPHOS activity, release of cytochrome c, and cellular apoptosis and necrosis. A reduction in complexes I and III activities in isolated hepatic mitochondria has been described in response to hydrophobic bile acids.

**NASH (Non-alcoholic steatohepatitis)-** The most common cause of asymptomatic transaminaea for more than six months, NASH, usually represents a part of spectrum of non-alcoholic fatty liver disease (NAFLD). The most important risk factors are obesity (prevalence 30 -100%), type 2 diabetes mellitus (75%), hyperlipidemia (20-92%), and female sex. NASH is considered a disease of two hits— steatosis due to increased delivery of fatty acids to liver followed by decreased intrahepatic clearance of fatty acids (due to decreased mitochondrial beta oxidation, impaired cholesterol esterification, or impaired VLDL export) with resultant lipid peroxidation, cytokine induction, and induction of fas ligand. All these lead to inflammatory response and fractional killing. Over a follow up period of 3.5-11 years, 28% had progression of liver damage, 99% had no change, while 13% had improvement or resolution of liver injury.

**Neonatal liver failure** - The classic presentation of respiratory chain disorders in childhood is severe liver failure in the first month of life, characterized by lactic acidosis, conjugated hyperbilirubinunemia with elevated (2 to 12 times) transaminases, coagulopathy, ketotic hypoglycemia, and renal tubulopathy. Liver biopsy shows microvesicular with or without macrovesicular steatosis, cholestasis and bile ductular proliferation. Periportal and centrilobular fibrosis may develop into nodular cirrhosis. Ultrastructural evidence of mitochondrial injury may be observed as swollen mitochondria, abnormal cristae, paracrystalline arrays, and a fluffy matrix, although normal mitochondrial morphology does not exclude these disorders. The hepatic activity of respiratory chain complex IV (commonest), I, III, and occasionally complex II is very low in these infants. Mutations in nuclear gene, BCS1L have also been reported.

Deficiencies of the respiratory complex enzymes have been associated with a later onset of recognizable liver disease in infancy and early childhood (between 2 months and 8 years of age). In most cases liver failure is preceded by neurological manifestation including ataxia and refractory partial motor epilepsy or multifocal myoclonus. A family history of an affected sibling has been noted in 50% cases.

**Mitochondrial DNA depletion syndromes -** Several infants have been described with severe or fatal liver disease caused by inherited mtDNA depletion syndromes (MDS), characterized by reduction in tissue-specific mtDNA copy number. Ultrastructurally, mitochondria show ‘oncocytic transformation’. A depletion to <10% of normal is required to produce a clinical syndrome. The severity of depletion correlates with the severity of tissue involvement and biochemical defects.

**Pearson’s marrow pancreas syndrome** - In this disorder, marked hepatomegaly, steatosis, hemosiderosis, and cirrhosis have been associated with liver failure and death in some cases by 3 months of age. Large scale heteroplasmic mtDNA rearrangements are constantly observed in affected and non-affected organs. The tissue distribution and relative proportion of abnormal mtDNA molecules appear to contribute to the phenotype.

**Navajo neurohepatopathy** - A recessively inherited sensorimotor neuropathy with progressive liver disease has been demonstrated in Navajo children; infantile and childhood forms are notable for their hepatic presentation with failure to thrive due to rapid development of liver failure. Liver histology shows portal fibrosis, micronodular cirrhosis, steatosis, pseudoacinar formation, multinucleated giant cells, cholestasis, and perportal inflammation. Non-specific mitochondrial changes like swollen mitochondria, ringed cristae are seen in several patients.

**Acute fatty liver of pregnancy (AFLP)** - In pregnancy,
hepatic metabolism of triglyceride and fatty acid increase greatly. Genetic abnormalities in fatty acid oxidation especially long chain 3-hydroxyacyl CoA dehydrogenase deficiency in the mother predispose her to the development of AFLP, which is associated with considerable risk of maternal and fetal morbidity and death.32

**DIAGNOSIS OF MITOCHONDRIAL DISORDERS**

Diagnosing a respiratory chain defect in patients with liver disease requires a high index of suspicion.33 In the following clinical circumstances a respiratory chain disease should be considered:

1) Association of neuromuscular symptoms with liver dysfunction,
2) Multisystem involvement in a patient with acute or chronic liver disease,
3) A rapidly progressive course of liver disease, particularly in the presence of lactic acidosis and ketonemia.

Laboratory findings suggestive of a respiratory chain disorder are listed in the Table 2.34

Searching for dysfunction or abnormal histology / biochemistry of the target organs is also important. Definitive tests include quantitation of enzymatic activity in the affected tissue, histopathology including histochemical stains for cytochrome c oxidase activity, genotyping for mtDNA or nuclear DNA mutations or deletions, and testing for mtDNA depletion.34 For secondary disorders, there are specific investigations for copper overload (serum copper, copper- binding proteins, urinary copper, hepatic copper), iron overload (serum ferrokinetics, hepatic iron), cholestasis, and NASH. A careful history will help in suggesting a diagnosis of Reye’s syndrome or a culprit drug intake.35

**TREATMENT**

Despite significant advances in our understanding of mitochondrial genetics, current treatment strategies for mitochondrial hepatopathies are generally supportive.35

**Medical management :** It includes the empiric supplementation with various ‘mitochondrial cocktails’ (that includes various antioxidants, vitamins, cofactors, and electron acceptors) (Table 3), supportive therapies, and avoidance of drugs and conditions known to have

### Table 2 : Laboratory tests for mitochondrial disorders

- **Plasma lactate/ pyruvate (L/P) molar ratio:** Often > 20 in respiratory chain disorders. Often < 10 in defects of pyruvate dehydrogenase. Plasma lactate typically > 2.5 mM (repeatedly). Oral glucose load (2 g/Kg): repeat plasma L/P testing each 15 minutes for 90 minutes.
- **Plasma ketone body (3-OH butyrate/ acetoacetate) molar ratio:** Often > 2 in OXPHOS defects. Often < 1 in disorders of pyruvate and Kreb’s cycle metabolism.
- **Plasma amino acid quantitation:** Can have elevations of alanine and proline in situations of lactic acidosis.
- **Plasma acylcarnitine profile:** To evaluate for fatty acid disorders. Can also measure total and free carnitine.
- **Urinary organic acids:** To evaluate for urine lactate, succinate, fumurate, maleate, 3-methyl glutaconate, and 3-methylglutarate by gas chromatography- mass spectroscopy.
- **Cerebrospinal fluid (CSF) studies:** L/P molar ratio. Ketone body molar ratio. Amino acid quantitation.
- **Skeletal muscle biopsy:** Light microscopy for presence of red ragged fibers. Electron microscopy for mitochondrial ultrastructure. Respiratory chain analysis (polarography with or without spectroscopy).

### Table 3 : Pharmacological treatment of mitochondrial disorders

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<th>(A) Electron acceptors and co-factors:</th>
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<tbody>
<tr>
<td>Coenzyme Q</td>
<td>Redox bypass of complex I</td>
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<tr>
<td></td>
<td>Free radical scavenger (antioxidant)</td>
</tr>
<tr>
<td>Idebenone</td>
<td>Redox bypass of complex I</td>
</tr>
<tr>
<td></td>
<td>Free radical scavenger (antioxidant)</td>
</tr>
<tr>
<td>Thiamine (vitamin Bₙ)</td>
<td>Cofactor of pyruvate dehydrogenase</td>
</tr>
<tr>
<td>Riboflavin (vitamin B₂)</td>
<td>Acts as flavin precursor for complexes I and II</td>
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<tr>
<td>Menadione (vitamin K₃)</td>
<td>Bypass complex III (with vitamin C)</td>
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<th>(B) Antioxidants:</th>
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<tbody>
<tr>
<td>Vitamin E (Tocopherol)</td>
<td>Antioxidant</td>
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<td></td>
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<tr>
<td>Vitamin C (Ascorbic acid)</td>
<td>Antioxidant</td>
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<th>(C) Other mechanisms:</th>
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<tr>
<td>Succinate</td>
<td>Donates electron directly to complex II</td>
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<tr>
<td>Carnitine</td>
<td>Replaces secondary carnitine deficiency</td>
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<tr>
<td></td>
<td>Ped: 50-100 mg/kg/day</td>
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<tr>
<td>Creatine monohydrate</td>
<td>Enhances muscle phosphocreatine</td>
</tr>
<tr>
<td></td>
<td>Ped: 0.1-0.2 gm/kg/day</td>
</tr>
<tr>
<td>Dichloroacetate</td>
<td>Reduces lactic acidosis by enhancing pyruvate dehydrogenase activity</td>
</tr>
<tr>
<td></td>
<td>Ped: 25-50 mg/kg/day</td>
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a detrimental effect. Hypermetabolic states (like exhaustive exercise, fever), infection, and drugs interfering with mitochondrial metabolism (like phenobarbitone, valproate, NSAIDs, aminoglycosides) should be avoided. Improved means of detecting early significant lactic academia and liver dysfunction may lead to timely discontinuation of the offending agent and prevention of more severe lactic acidosis, pancreatitis and liver failure. Vitamin E has provided significant protection against hydrophobic bile acid toxicity in an in vivo rat model. In addition to copper and iron chelation therapy for Wilson’s disease and iron-overload conditions respectively attempts to reduce oxidative stress (e.g. with antioxidants) in the liver could potentially help protect the mitochondria from injury in these disorders. Aspirin should not be used in children with viral fever. Possible interventions in humans with cholestasis to improve mitochondrial function and prevent triggering of injurious pathways are under investigation. Treatment with membrane-stabilizing agent like ursodeoxycholic acid (UDCA) has a role to play. Treatment options for NASH are limited and include weight reduction, UDCA, and liver transplantation (in those with cirrhosis). Treatment based on pathogenic mechanisms of NASH includes inhibitors of macrophage activation, PARP inhibitors, dietary modification, insulin sensitizers, anti-obesity drugs, and anti-lipid agents.

Liver transplantation: Several patients with defects isolated to the liver have now successfully undergone liver transplantation with excellent long term outcomes and no extra-hepatic disease expression. Extrahepatic disease, especially neurologic disease, should be ruled out beforehand but at times may be difficult. For patients with acquired mtDNA depletion caused by nucleoside analogues, successful liver transplantation has been performed without recurrence of disease, as long as the offending agent has been discontinued.

REFERENCES


Announcement

Indo-Australian Critical Care Training Programme

Indo-Australian Critical Care Training Programme - 2005 (A Certificate Course organised by the College of Critical Care Medicine and Critical Care Department of AMRI Hospitals under auspices of the Critical Care Education Foundation) 9th to 11th December, 2005 at Science City Auditorium, Saltiake, Kolkata.

Registration fees
Conference - Rs. 2000/- (PG students) & Rs. 2500/- (regular) for Workshop Rs. 1000/- (Pg Student) & Rs. 1500/- (regular).

All payment to be made by Demand Draft only, in favour of "Critical Care Education Foundation" payable at Mumbai.

For details contact Dr. SK Todi, Co-ordinator, AMRI Hospitals, P-4 & 5, CIT Scheme, LXXII, Block - A, Gariahat Road, Kolkata-700 029.
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