Mitochondrial Medicine

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Abstract
Mitochondrial diseases are extremely heterogeneous multisystem disorders predominantly affecting tissues or organs with high oxygen consumption like skeletal muscles, brain, endocrine glands, myocardium, eyes, ears, intestines, liver, kidneys, and bone marrow. Although various clinical syndromes have been described, they frequently overlap and there is no diagnostic gold standard to identify all. It is difficult to chart the future of an affected individual as also to predict the response to treatment which is mostly supportive and symptomatic. The rapidly increasing understanding of the pathophysiologic background of mitochondrial disorders may facilitate diagnostic approach and open perspectives to curative therapies. With the coming of age for mitochondrial medicine, it is now appropriate that physicians keep themselves well-acquainted with the recent developments in this expanding field of biomedical research.

Introduction
Since the end of the 1980s, key discoveries have been made which have significantly revived the scientific interest in a cell organelle named mitochondria, that has been studied continuously and with steady success for the last 100 years. It has become increasingly evident that mitochondrial dysfunction contributes to a variety of human disorders, ranging from neurodegenerative and neuromuscular diseases, obesity and diabetes to ischemia-reperfusion injury and cancer. Although the production of energy is indeed the primary function of mitochondria, attention has also been directed toward their role in producing reactive oxygen and nitrogen species and the subsequent deleterious effects of these intermediates. Understanding the role that mitochondrial dysfunction plays in the pathogenesis of common disorders, provided unique insights into a number of diseases and offered hope for potential new therapies.

Structural Assembly of Mitochondria
Mitochondria are intracellular ovoid organelles with a transverse diameter of 0.1-0.5 µm and varying length. They reside between the myofibrils, subsarcolemmally, near the nucleus or near the motor end plate (Figure 1). Their number per cell ranges from none (erythrocytes) to 10,000 (striated muscle cells), and increases with the amount of substrate and oxygen utilization a particular cell require.

Mitochondria are built up of four compartments (Figure 1):

- a. Within the outer lipid layer or closely associated with it reside proteins like TOM/TIM complex protein import system (for entry of nuclear-encoded proteins), porine (voltage-dependent ion channels), NADH cytochrome-b, reductase, palmitoyl-CoA-synthetase, carnitine-palmitoyltransferase I, and mono-amine-oxidase. This layer is permeable to molecules <10,000 Dalton.
- b. The inner lipid membrane is folded and impermeable to most molecules and proteins (except unloaded molecules like O2, CO2, and H2O). It is built up mostly of proteins, devoid of cholesterol, with abundant cardiolipin. Embedded in the inner membrane are the four respiratory chain (RC) complexes, complex V (ATP-synthase), ubiquinone, and carnitine-palmitoyltransferase II. There are carriers for anions, cations and redox equivalents including that responsible for the exchange of adenosine-diphosphate (ADP) and adenosine-tri phosphate (ATP).
- c. Within the intermembrane space reside the adenylate-kinase, creatine-phosphokinase and cytochrome-c; the last-mentioned one initiates apoptosis if released in the cytosol.
- d. Within the matrix a large number of enzymes, proteins and peptides including RC complexes, DNA-polymerases, chaperones, mRNAs, tRNAs, and the mitochondrial DNA (mtDNA) are located.

Biological Role of Mitochondria
Mitochondria subserve five functions in humans:

1. Mediation of intermediary metabolism - Mitochondria harbour the β-oxidation, citrate cycle, degradation of amino acids, parts of the haem-biosynthesis, parts of steroid metabolism, parts of the uric acid cycle, and the pyruvate-dehydrogenase complex responsible for the decarboxylation of pyruvate.

2. Provision of ATP - The principal function of mitochondria is to produce energy in the form of ATP. The core of the RC and oxidative phosphorylation (OXPHOS) are the five multunit complexes I-V. Electrons from various substrates pass along the chain, providing energy to pump protons...
Mitochondrial Diseases

Mitochondrial diseases are usually multisystem disorders, which predominantly manifest in tissues or organs with high oxygen consumption like skeletal muscles, brain, endocrine glands, myocardium, eyes, ears, intestines, liver, kidneys, and bone marrow. Less than 5% are due to mtDNA mutations while the rest are due to mutations in nDNA. Mitochondrial diseases may be due to inherited (frequently point mutations) or acquired (commonly deletions and insertions) mutations. Factors which determine whether mutations will be pathologically relevant are: (i) heteroplasmy rate (ii) threshold effect (iii) correlation of the mutation with a biochemical defect, (iv) transferability of the defect and (v) whether there is alteration in conserved base pairs or amino acids. Mitochondrial diseases caused by nDNA mutations follow a Mendelian pattern of inheritance.

Mitochondrial diseases are classified according to genetic or biochemical criteria (Table 1). In children, approximately one-third of the inherited metabolic disorders are attributable to mitochondrial dysfunction. The prevalence of mtDNA mutation in adults is estimated to be 1,5000. The onset ranges from early embryogenesis to late adulthood. A striking feature is their clinical heterogeneity, ranging from single organ involvement to severe multisystem disease. There are a number of well recognized syndromes. A high index of suspicion is required if a common disease has some atypical features, if several organ systems are involved, if there are recurrent flare-ups in a chronic disease.

While taking the history issues such as maternal health, obstetric history, family history of neonatal or childhood deaths, deafness, diabetes, cardiac disease, visual impairment, and developmental delay should be particularly addressed. Possible manifestations on visceral organs are summarized below:

Neuromuscular system – facial dysmorphism, mental retardation, dementia, impaired visual acuity, visual field defect, diplopia, nystagmus, hyperacusis, deafness, dystarthisia, dry mouth, reduced gag reflex, exaggerated masseter reflex, gaze paresis, ophthalmoparesis, weakness, wasting, hypotonia, reduced deep tendon reflexes, long tract signs, fasciculation, myoclonus, cogwheel rigidity, dystonia, ataxia, brady/ dysdiadochokinesia, sensory and gait disturbance.

Eye - pigmentary retinopathy, optic atrophy, cataract, and glaucoma.

Endocrine system – short stature, developmental delay, polyphagia, failure to thrive, hypopituitarism, diabetes mellitus, diabetes insipidus, hypoglycemia, thyroid and parathyroid dysfunction, amenorrhoea, hypogonadism, delayed puberty

Heart – cardiomyopathy, rhythm abnormalities, left ventricular hypertrabeculation

Gastrointestinal – paradontosis, dysphagia, gastrointestinal dysmotility, pseudoobstruction, recurrent vomiting, hepatopathy (acute liver failure), recurrent pancreatitis, villus atrophy, malabsorption, diarrhea, weight loss, anorexia

Kidneys – renal cysts, tubulopathy, Toni-Debre-Fanconi syndrome

Blood – anemia, leucopenia, thrombocytopenia, eosinophilia

There may be an increased sensitivity to general anaesthetic agents like etomidate and thiopental. In addition, certain drugs impair mitochondrial functions like aspirin, alcohol, valproate, barbiturates (block complex I), tetracyclines, chloramphenicol (reduce mitochondrial protein synthesis), doxorubicin and zidovudine (causes mtDNA depletion), while vitamin C and E exert protective effects. Thus a thorough drug history is important. Recently, steroid-induced myopathy has been
proposed to be a mitochondrial disorder.\textsuperscript{21}

The following is the case illustration of five important mitochondrial disorders:

\textbf{Case 1} : A two and a half year old boy presented with acute bronchopneumonia for two weeks complicated by acute respiratory failure and had to be mechanically ventilated. The mother has a history of two abortions prior to the birth of this child and unexplained neonatal death on the matriarchal lineage. His birth weight was 3 kg and there was no history suggestive of perinatal asphyxia. He had two sisters who were healthy. The child was asymptomatic till 9 months of age when he developed fever and seizures and was administered symptomatic treatment. He had attained standing with support, sitting without support and bisyllables by that age. One and a half months later, he was noticed to have difficulty in speaking and subsequent arrest in gaining further developmental milestones in all domains. There has also been a loss of personal-social and language mile stones since then and he has also failed to thrive.

On examination, the child was febrile and chest auscultation revealed bilateral crepitations and rhonchi. On neurological examination, he was conscious but not oriented, left pupil was dilated and not reacting, there was hypertension (lower limbs more than upper limbs and left side more than right side), involuntary movements (bilateral choreothetoid movements and myoclonic jerks), exaggerated deep tendon reflexes and equivocal plantar reflexes. He did not fix his eyes or follow light. He also had a palpable liver of 3 cm below the costal margin. Fundus examination and visual evoked potentials were normal.

Magnetic resonance imaging (MRI) of the brain revealed diffuse cerebral and brainstem atrophy. There were bilateral symmetric T2 hyper intense lesions involving the pulvinar and dorsal aspects of the thalami and in the parietal, occipital and posterior temporal regions. The involved regions showed restriction of diffusion on diffusion-weighted imaging. CSF analysis was normal except for a slightly elevated lactate. 2, 4 DNPH (dinitro-phenylhydrazine) test was positive on metabolic screening. This observation in the absence of neuro-infection was suggestive of a mitochondrial disorder - Leigh Syndrome.

\textbf{Case 2} : A 19-year-old male presented to the Ophthalmologist with progressive drooping of both upper lids since three years, associated with limited movements of eyeball in all directions. He also had difficulty in hearing particularly at higher frequencies. Cranial CT and MR imaging were normal. \textit{Window defects} were also had difficulty in hearing particularly at higher frequencies. 

Diagnosis

The diagnostic approach has to be individualized incorporating clinical, electrophysiological, imaging, histological, biochemical and genetic investigations. Myopathy with or without lactic acidosis is the most common presenting feature.\textsuperscript{4} Combination
Table 2: Treatment modalities in mitochondrial disorders

**General measures**
- Tailor individual therapy to optimally meet the patients need
- Dietary measures [no fasting, ketogenic dietary (65% fat) in PDC deficiency, no glutamate, reduction of fat and simultaneous increase in carbohydrates (except PDC deficiency)]
- Physical exercise only below the maximal individual limit (avoidance of overexertion)
- Avoidance of mental and physical stress (plenty of sleep), cold and heat stress, including direct exposure to sunlight, infections, fasting, alcohol, smoking
- Physical therapy, orthosis, crutches, braces, wheel chair for motor problems

**Medications**
- Coenzyme-Q (5-15 mg/kg/day), idebenone (coenzyme analogue) (90-225 mg/day)
- L-carnitine (30-100 mg/kg/day), acetyl L-carnitine (250-1000 mg/day)
- Vitamin C (100-1500 mg/day), Vitamin D, Vitamin E (200-1200 IU/day), Vitamin K3 (5-30 mg/day), thiamine (50-100 mg/day), nicotinamide (50-100 mg/day), riboflavin (50-200 mg/day), lipoic acid (180-300 mg/day), β-carotene (10,000 IU/day), biotin (2.5-10 mg/day)
- Selenium (25-50 µg/day), Calcium, phosphate
- Succinate (6 g/day), creatine-mono-hydrate (5 g/day), uridine, citrate, dicholoroacetate (reduces serum lactate)

**Specific measures**
- Therapy for seizures, dementia, migraine, spasticity, dystonia, Parkinsonism, pain, cramps, muscle stiffness, myotonia, control of diabetes
- Symptomatic/supportive therapy for renal insufficiency, hepatic failure, cardiac impairment, medical nutrition therapy and drug therapy for osteoporosis and hyperlipidemia
- Hormone replacement therapy
- Prescription of a hearing device
- Surgical correction of ptosis, cataract, glaucoma
- Percutaneous enterogastrostomy

of clinical features like deafness, cardiomyopathy and diabetes together with encephalopathy and myopathy are highly suggestive and should be regarded as red flags. Exercise intolerance is the clinical hallmark of RCD.

The most important biochemical parameters are serum and cerebrospinal fluid lactate and pyruvate. Both are frequently increased at rest. The serum lactate/ pyruvate ratio is increased >20 in RCDs and citrate cycle defects, but <10 in PDC defects. Creatine-kinase levels are often elevated with highest values found in mtDNA depletion syndromes. There is an exaggerated accumulation of extracellular lactate during aerobic exercise compared to healthy subjects (‘lactate stress test’). The ‘ischemic forearm test’ is as sensitive as muscle biopsy and based on decreased oxygen extraction from capillary blood, resulting in high O2 saturation in venous effluent blood from working muscles.

Muscle biopsy is the most helpful diagnostic procedure for evaluation. Muscle tissue should be investigated for: i) Routine light microscopy including modified Gomori trichrome stain (ragged-red fibers), ii) Immunohistochemistry including COX (cytochrome oxidase), succinate dehydrogenase (ragged-blue fibers), NADH, ATPase, periodic acid Schiff (PAS), lipid stain, iii) Electron microscopy to view the mitochondrial structure (whirled mitochondria, megalomitochondria, and paracrystalline inclusions), iv) Biochemical investigations by spectrophotometry (usualy performed in tissue homogenates) include analysis of the individual or group complex activity, the β-oxidation spiral and carnitine/ acylcarnitine transport capacity, v) Polarography determines the RC activity, the OXPHOS activity, the integrity of mitochondrial membranes, and the efficiency of substrate transport.

Genetic testing should be carried out only if the clinical features are highly suggestive of one of the classical syndromes. Sequencing of the entire mtDNA should be carried out only from muscle mtDNA. Evidence of new mtDNA mutations is best provided by single-fiber polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis.

**Diagnostic scores**
- Wolf and Smeitink proposed a scoring system (mitochondrial disease criteria, MDC), which relies upon symptoms, metabolic and imaging findings, skeletal muscle morphology, biochemical investigations, and genetic data. Clinical criteria are divided into three main groups: skeletal muscle, CNS, and multisystem. Biochemical investigations include oxidation rates of 14C labeled substrate, ATP and phosphocreatine production rate and activity of single RC complexes. All available information is scored and the results assigned to one of the four levels: ‘unlikely’, ‘possible’, ‘probable’, or ‘definite’. Presence of ragged-red fibers, mitochondrial abnormalities on electron microscopy, or a known pathogenic mutation is regarded as ‘definite’ criteria. A novel heteroplasmic mutation in symptomatic individual, or abnormalities found on enzymology or polarography are regarded ‘probably’ diagnostic. First degree relatives of a diagnosed case have ‘possible’ disease.

**Therapeutic Manipulations**
- Treatment for the large part is supportive and symptomatic (Table 2). No treatment is able to reverse the already sustained damage. An individualized treatment is more effective than empirical approach. A group of Specialist from different subspecialties of medicine should be involved.
- Generally non-specific pharmacological treatment is of limited benefit and short-lived, suggesting a strong placebo effect. The effectiveness of treatment varies from patient to patient, depending on the particular disorder and its severity. Mild diseases tend to respond better to therapy than severe disease.

Mitochondria-specific delivery of drugs is still in its infancy. Vesicular carrier like self-assembling mitochondriotropics dequalinium (DQA) has shown some promise in delivery of plasmid DNA to mitochondria in living mammalian cells.

**Genetic counseling**
- Genetic counseling is difficult because of the genetic heterogeneity, uncertain heteroplasmy rates, and threshold levels. However, the following comments can be made with confidence.
  - Most cases of mtDNA mutations are sporadic cases
  - Point mutations are more commonly transmitted than single gene mutations
  - The risk of having an affected child increases with the level of heteroplasmy in mother
  - Males with mtDNA mutations will only exceptionally transmit the disease
v. Expression and severity vary within a given family
vi. A negative family history does not exclude the familial occurrence
vii. Sampling of any fetal tissue is ought to provide a reliable indicator of the overall mtDNA mutation load

**Conclusion**

Primary defects in mitochondrial function are implicated in a number of human diseases and the list continues to grow. The field’s dramatic expansion reflects growth of knowledge in three areas: (1) characterization of mitochondrial structure and function, (2) elucidation of the steps involved in mitochondrial biosynthesis, and (3) discovery of specific mitochondrial DNA. The clinical manifestations are protean, most often involving skeletal muscle and central nervous system. Manifestations of both the primary and secondary mitochondrial diseases are thought to result from the production of oxygen free radicals. With increased understanding of the mechanisms underlying the mitochondrial dysfunctions has come the beginning of therapeutic strategies, based mostly on the administration of antioxidants, replacement of cofactors, and provision of nutrients. At the present accelerating pace of development of what may be called ‘mitochondrial medicine’, much more is likely to be achieved in near future.

**References**

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