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CURRENT UNDERSTANDING OF DIAGNOSIS AND TREATMENT OF RARE MITOCHONDRIAL DISORDERS

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ABSTRACT

Each of our cells contains on an average 500 to 2,000 little "power factories" called mitochondria that are responsible for supplying our energy needs. Approximately 1000 different proteins in mitochondria and defects in many such proteins can be characterized and described under the heading `metabolic diseases', or inborn errors of metabolism. Mitochondrial disorders are a clinically heterogeneous group of disorders that arise as a result of dysfunction of the mitochondrial respiratory chain or electron transport chain. The manifestations of mitochondrial disorders are extremely diverse; include numerous symptoms of variable severity, and affect many different organs of the body such as brain, kidneys, muscles, heart, eyes, ears, etc. Many mitochondrial disorders are so new that they have not yet been mentioned in the medical textbooks or in to the medical literature. Mitochondrial disorders are caused by mutations in either mitochondrial DNA (mtDNA) or nuclear DNA. Elevated lactic acid or lactate to pyruvate ratio (>20:1) in blood or cerebrospinal fluid (CSF) is a common sign of mitochondrial dysfunction. Muscle biopsy is the gold-standard for the diagnosis of many mitochondrial disorders and requires specialized microscopic analyses and biochemical tests. Laboratory studies typically include: blood tests, brain MRI or CT scans, heart tests (electrocardiogram and echocardiograms), ophthalmological and neurological evaluations, and hearing tests. Finally, genetic analysis of blood, urine, or muscle is performed to pinpoint the exact mutation responsible for a specific disease. Treatment of mitochondrial disorders is limited. Therapies to treat specific symptoms and signs of mitochondrial disorders are very important. This article provides a brief summary of our present knowledge and understanding of mitochondrial disorders.

INTRODUCTION

Mitochondria are intracellular organelles that produce energy in the cell. Primordial eukaryotic cells were initially anaerobic, before they developed symbiotic relationship with bacteria that use oxygen and eventually these bacteria evolved into mitochondria.[1] Human body contains approximately 250 different cell types, whose gene expression varies in each cell type through selective transcription and are tailored to meet specialized needs.[2] In the same manner, mitochondrion is tailored to meet the energy demands of the various cell types.[3] Cells have very high to very low number of mitochondria, depending upon their energy requirement.

Cone photoreceptor cells of human eye have maximum number of mitochondria to meet the higher demand for metabolic energy associated with photo transduction.[4] Mitochondria comprise ~80% of intracellular volume of cone cells. Similarly, in extra-occular muscles, mitochondria comprise ~60% of intracellular volume. In cardiac muscle cells, mitochondria comprise ~40% of intracellular volume. Some cells have very few mitochondria and some are completely lacking. Thrombocytes have only 2-6 mitochondria.[5] Although the pre-erythroblast has mitochondria, mature erythrocyte does not have mitochondria.

Mitochondria are comprised of outer, inner membranes and cytoplasm called matrix. Inner membrane has series of protein complexes, known as electron transport chain. These complexes require ubiquinone and cytochrome c cofactors. Area between two membranes harbours enzymes involved in fatty acid transport. The matrix has the enzymes involved in beta oxidation of fatty acids.[6] Although, mitochondria are having their own genome, nuclear genome regulates the biogenesis of mitochondria and encodes 99% of its proteins.[7] Biogenesis of mtDNA requires nuclear genes, viz., DNA polymerase gamma (polG) and DNA helicases.[8] Defects in these enzymes cause mtDNA depletion and multiple deletions. Hence, the activities of mitochondrial components depend upon nuclear as well as mtDNA.[9, 10] Mitochondrial genome has only 37 genes, out of which 13 encode 13 enzymes out of 90 involved in respiratory chain, 2 rRNAs and 22 tRNAs.

Thus majority of enzymes of respiratory chain are encoded by nuclear genome.

Further, replication of mtDNA mostly depends upon factors encoded by nuclear DNA, indicating its control over mtDNA and its proteins. Thus, an intricate inter-genomic communication plays a role in biogenesis of mitochondria and mitochondrial DNA. Mitochondrial genome is circular and has 16,569 nucleotides, with two hyper variable regions, cytochrome b region, several subunits ND1 to ND6, complex III, IV, ATP6 and ATP8.[11] HVR I and II are useful in tracing the maternal ancestry in population genetics and cytochrome b region is useful in species identification and forensic studies. Because mtDNA have a high mutation rate and lack of repair mechanisms, once a mutation occurs in mtDNA it is permanent.[12] Hence, we can find many mutations, all over the circular mtDNA. Furthermore, mitochondrial genes are not having introns and follow non universality of genetic code.[13]

MITOCHONDRIAL DISORDERS

Mitochondrial disorder can refer to the shutdown of some or all the mitochondria that lead to cutting of essential energy supply to the cell or tissues.[14] Initially mtDNA mutations were thought to be the reason to cause mitochondrial disorder.[15-18] Later, knowing about the control of nuclear DNA over mitochondrial DNA and mitochondrial biogenesis, researchers are looking into the nuclear DNA.[19-22] Mitochondrial disorders may also be the result of acquired mitochondrial dysfunction due to drugs, infections and environmental factors.[23] Mitochondrial disorder can be sporadic or inherited. mtDNA disorders show maternal inheritance because embryo acquires mitochondria only from oocyte due to exclusion of sperm cell mitochondria that are located in its midpiece.[24] If the mother is having mitochondrial disorder, it will be transmitted to both sons and daughters. But sons cannot transmit to their progeny, as the daughters do. If nuclear genes are involved, the inheritance pattern may be autosomal dominant or recessive.[25,26] In the absence of solid genotype-phenotype correlation, in some cases correlation can be identified, if nuclear genes coding mitochondrial proteins are involved. Mitochondrial Neurogastrointestinal Encephalopathy (MNGIE) is one of the important rare mtDNA disorders.[27,28] This disease is characterized by progressive gastrointestinal dysmotility and cachexia manifesting as early satiety, nausea, dysphagia, gastroesophageal reflux, postprandial emesis, episodic abdominal pain and/or distention, and diarrhea; ptosis/ophthalmoplegia or ophthalmoparesis; hearing loss; and demyelinating peripheral neuropathy manifesting as paresthesias (tingling, numbness, and pain) and symmetric and distal weakness more prominently affecting the lower extremities.[29]

VARIATIONS IN PHENOTYPIC EXPRESSION

The involvement of mtDNA and nuclear DNA mutation property, heteroplasmy, threshold effect (bottleneck phenomenon), mitotic potential of the tissue, energy demand of tissue and age related changes in the mitochondria may greatly affect the phenotypic expression of mitochondrial disorders.[30] If the nuclear DNA mutations are involved, disease manifests in early childhood and have more severe and diffuse expression. Unlike nuclear DNA mutations, mitochondrial mutations manifest the disease in adulthood with more indolent and mosaic fashion.[31,32] The increasing clinical and genetic heterogeneity of mitochondrial disorders that are reported in recent literature, reflect the above principle (**Table 1**). Presence of both wild and mutant mtDNA in cell is called as heteroplasmy. Because of heteroplasmy, proportion of mutant mtDNA differs in the different tissues or even in cells of same tissue. Disease can be expressed only when the mutant mtDNA reaches to certain threshold, which depends on the energy metabolism of the cell.[33,34] If the cell is dividing mitotically, the mutant and wild type mitochondria will be randomly segregated into daughter cells. In case of neurons and muscle cells, which are not undergoing mitosis, mutations accumulate.[35] Age related changes in mitochondria, that include damage to mtDNA by free radicals, decrease in the efficiency of Krebe's cycle, altered response to long term energy demands, respiratory chain defects due to energy alterations and decreased membrane fluidity contribute to the phenotypic expression of mitochondrial disorders.[36-38]

DIAGNOSIS

There is no definite diagnosis for mitochondrial disorders. After taking the family history and clinical evaluation to identify recognizable syndromes, minimally invasive investigations like imaging, blood and urine chemistry or invasive investigations such as biochemical, histo-chemical and molecular studies on the biopsy sample collected from liver, skin and muscle may be performed (**Table 2**).[39-42] The biochemical tests include lactate and

Table 1: Overview of Clinical and Genetic Features Associated with Mitochondrial Disorders.

S.No.	Disorder	Age of Onset	Key Clinical Features	Gene Implicated/ Inheritance Pattern	Diagnosis	Treatment	Reference
1	Progressive Infantile Poliodystrophy (Alpers disease)	1-5 years	Seizures, dementia, cerebral degeneration, and liver dysfunction	POLG/ Autosomal recessive	DNA Mutation analysis	Anticonvulsants and Physiotherapy.	86, 87
2	Lethal infantile cardiomyopathy (Barth Syndrome)	Variable	skeletal myopathy, cardiomyopathy and neutropenia	TAZ/ X-linked recessive	Levels of 3-methylglutaconic acid in urine	Diet supplementation with L-carnitine or Oral pantothenol.	88, 89
3	Carnitine acylcarnitine translocase deficiency	Neonates	convulsions, hypothermia, encephalopathy, cardiomyopathy and liver dysfunction	SLC25A20/ Autosomal recessive	Enzyme assay or DNA analysis	Carnitine and a low -fat diet supplemented medium-chain triglycerides.	90
4	Carnitine deficiency	Neonates	Cardiomyopathy, failure to thrive, encephalopathy, skeletal myopathy	SLC22A5/ Autosomal recessive	Enzyme assay or DNA analysis	Diet supplementation with L-Carnitine	91,92
5	Cerebral Creatine Deficiency Syndrome	Infants to variable age	Mental retardation, expressive speech and language delay, autistic like behaviour and epilepsy	GAMT & AGAT / autosomal recessive; SLC6A8/ X-linked	Enzyme assay or DNA analysis	Diet supplementation with L-Carnitine	93, 94
6	Coenzyme Q10 Deficiency	Infants	Encephalomyopathy, nephropathy, cerebellar ataxia, and isolated myopathy and recurrent myoglobinuria	Probably autosomal recessive	Estimation of Co-enzyme Q10 level through HPLC	Administration of Co-enzyme Q10	95
7	Complex-1 or NADH dehydrogenase deficiency	Infants to adults	Myopathy, Mitochondrial encephalomyopathy and fatal infantile multisystem disorder	Gene families of NDUFS, NDUFB, NDUFA and MTND/ Maternal or Autosomal recessive or X linked	Enzyme assay	riboflavin, thiamine, biotin, co -enzyme Q10, carnitine, and the ketogenic diet	96
8	Complex-2 or Succinate dehydrogenase deficiency	Infants to adults	Encephalomyopathy, developmental delay, hyoptonia, respiratory failure, ataxia, myoclonus.	SDHA, SDHAF1/ autosomal recessive	Enzyme assay	No effective treatment	97, 98
9	Complex-3 or Ubiquinone-cytochrome c oxidoreductase deficiency	Infants to adults	Fatal infantile encephalomyopathy and infantile histiocytoid cardiomyopathy.	UQCR gene family or MT-CYB / Probably autosomal recessive/ Maternal	Enzyme assay	No effective treatment	99, 100
10	Complex-4 or Cytochrome c oxidase deficiency	Infants to 2 years of age	Encephalomyopathy and myopathy	COX gene family/ Autosomal recessive	Enzyme assay and histopathology for ragged-red fibers	No effective treatment	101

S.No.	Disorder	Age of Onset	Key Clinical Features	Gene Implicated/ Inheritance Pattern	Diagnosis	Treatment	Reference
11	Complex-5 or ATP synthase deficiency	Infants to 10 years	Myopathy, hypotonia, hepatomegaly, facial dysmorphism and microcephaly	ATPAF2, TMEM70, ATP5E, ATP5A1/ *maternal inheritance*	Assaying ATP synthesis in cultured skin fibroblasts	No definitive treatment	102
12	Chronic Progressive External Ophthalmoplegia Syndrome	Before 20 years age	Dysfunction of the central nervous system, visual myopathy and retinitis pigmentosa.	mtDNA deletions and point mutations/ maternal inheritance	Muscle biopsy to visualize "ragged red fibers". DNA analysis	No definitive treatment, surgical intervention for drooping eyelids.	103
13	Carnitine Palmitoyl Transferase -1 deficiency	8 to 18 months	Enlarged liver, recurrent Reye-like episodes triggered by fasting or illnesses.	CPT1A/ Autosomal recessive	Enzyme assay, DNA analysis	Medium-chain triglycerides	104
14	Carnitine Palmitoyl Transferase -2 deficiency	Infants and 15 to 30 years	Myopathic, Reye-like syndrome, hepatomegaly, hypoglycemia, and cardiac arrhythmia.	CPT2/ Autosomal recessive	Enzyme assay, DNA analysis	High carbohydrate, low-fat diet	105
15	Kearns-Sayre Syndrome	Before 20 years age	Chronic progressive external ophthalmoplegia, pigmentary retinopathy, cardiac conduction defects.	mtDNA deletions/ Maternal	Muscle biopsy to visualize "ragged red fibers", DNA analysis and Lactic and pyruvic acid levels	Coenzyme Q10, insulin, cardiac drugs and surgical intervention for drooping eyelids.	106
16	Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL)	Infants, children and adults	slowly progressive cerebellar ataxia and spasticity with dorsal column dysfunction	*DARS2* / autosomal recessive	DNA analysis, brain and spinal cord MRI	Corticosteroids have shown relief in bladder symptoms	107, 108
17	Long-Chain Acyl -CoA Dehydrongenase Deficiency (LCAD)	Infants	Failure to thrive, hepatomegaly, cardiomegaly and metabolic encephalopathy	*ACADL* / Autosomal recessive	DNA analysis	High carbohydrate-low fat diet, medium-chain fatty acids. Carnitine or riboflavin supplementation.	109
18	Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)	Infants	Encephalopathy, liver dysfunction, cardiomyopathy and peripheral neuropathy	*HADHA*/ Autosomal recessive	DNA analysis, Fatty acid oxidation probe test	High carbohydrate-low fat diet, medium-chain fatty acids. Carnitine or riboflavin supplementation.	110

S.No.	Disorder	Age of Onset	Key Clinical Features	Gene Implicated/ Inheritance Pattern	Diagnosis	Treatment	Reference
19	Leigh Syndrome	Infants or childhood	Seizures, hypotonia, poor motor function, ataxia. Visible necrotizing lesions on the brain MRI scan	BCS1L, COX10, NDUF gene family/ Autosomal recessive/X-linked recessive	DNA analysis, lactic acidosis or acidemia and hyperalaninemia	Thiamine, coenzyme Q10, riboflavin, biotin, creatine, succinate, and idebenone. Dichloroacetate is being used in some clinics.	111
20	Luft Disease or Nonthyroidal hypermetabolism	Childhood	Hypermetabolism, hyperthermia, polyphagia, polydipsia, and resting tachycardia.	Unknown inheritance	Muscle biopsies showed ragged red fibers	Vitamins C, E, K and Coenzyme Q10, high calorie diet.	112
21	Glutaric aciduria type 2 or Multiple Acyl-CoA Dehydrogenase Deficiency (MADD)	Neonates and childhood to adulthood	Respiratory distress, muscular hypotonia, hepatomegaly, hypoglycemia, encephalopathy, seizures and heart failure.	ETFDH, ETFA, ETFB/ Autosomal recessive	Enzyme assay for short chain dicarboxylic acids in urine	Low fat and low protein diet. Coenzyme Q10 and Riboflavin	113, 114
22	Medium-Chain Acyl-CoA Dehydrongenase Deficiency (MCAD)	Infants and young children	episodes of encephalopathy, enlarged and fatty degeneration of the liver, and low carnitine in the blood.	*ACADM* / Autosomal recessive	Enzyme assay for plasma acylcarnitin, urine organic acid and acylglycine analysis	High carbohydrate-low fat diet, medium-chain fatty acids. Carnitine or riboflavin supplementation.	115
23	Mitochondrial Encephalomyopathy Lactic Acidosis and Strokelike Episodes (MELAS)	between the ages of 2 and 15	Seizures, stroke-like episodes with focused neurological deficits, recurrent headaches and cognitive regression.	mtDNA point mutations/ Maternal	Elevated serum lactate during acute episodes. Respiratory enzyme defects in skeletal muscle	CoQ10, creatine, phylloquinone, other vitamins and anticonvulsants.	116
24	Myoclonic Epilepsy and Ragged-Red Fiber Disease (MERRF)	in childhood	Myoclonus, epilepsy, progressive ataxia, muscle weakness and degeneration, deafness, and dementia	mtDNA point mutations/ Maternal	Histopathology for ragged red fibers, strong reaction for SDH and COX deficiency.	CoQ10, creatine, phylloquinone, other vitamins and anticonvulsants.	117
25	Mitochondrial Recessive Ataxia Syndrome (MIRAS)	Children to adults	Encephalopathy, neuropathy, refractory epilepsy, ataxia and hepatopathy.	POLG/ Autosomal recessive inheritance	Muscle biopsy and DNA analyses	Ketogenic diet, vitamins and Anticonvulsants. Mitochondrion-toxic drugs should be avoided.	118, 119

S.No.	Disorder	Age of Onset	Key Clinical Features	Gene Implicated/ Inheritance Pattern	Diagnosis	Treatment	Reference
26	Mitochondrial DNA Depletion	Neonates to 20 year of age	Myopathic, encephalomyopathic, hepatocerebral and neurogastrointestinal presentations	TK2, SUCLA2, SUCLG1, RRM2B, DGUOK, TYMP, and POLG / Autosomal recessive	Histopathology for ragged red fibres and SDH. Quantitative real time PCR for mtDNA content in muscle, fibroblasts, blood and liver.	Ketogenic diet, vitamins and Anticonvulsants. Mitochondrion-toxic drugs should be avoided.	120, 121
27	Myoneurogasto intestinal Disorder and Encephalopathy (MNGIE)	Infants to adults	Severe gastrointestinal dysmotility, cachexia, ptosis, external ophthalmoplegia, sensorimotor neuropathy and asymptomatic leukoencephalopathy.	TYMP/ Autosomal recessive	Assay for plasma thymidine and deoxyuridine concentrations	Mitochondrion-toxic drugs should be avoided. Drugs primarily metabolized in liver should be used cautiously.	122
28	Neuropathy, Ataxia, and Retinitis Pigmentosa (NARP)	childhood or early adulthood	sensory neuropathy, muscle weakness, ataxia, dementia, seizures, hearing loss and cardiac conduction defects	mtDNA point mutations / maternal inheritance	Lactate in blood and CSF, Alanine in plasma. Cerebellar atrophy on MRI	Antioxidants help in symptomatic relief.	123
29	Pearson Syndrome	Infants	sideroblastic anemia, exocrine pancreas dysfunction, steatorrhea, pancreatic fibrosis and insulin-dependent diabetes.	mtDNA deletions/ maternal inheritance	Ring sideroblasts are erythroblasts with iron-loaded mitochondria visualized by Prussian blue staining.	Administration of coenzyme Q_{10} and L -carnitine, physical and occupational therapy.	124
30	Pyruvate Carboxylase Deficiency	Infants to adults	Developmental delay, recurrent seizures, and metabolic acidosis.	*PC* /autosomal recessive	Enzyme assay in fibroblasts, lactic academia and amino acids in serum and urine.	Hydration and correction of the metabolic acidosis. Supplementation of citrate, aspartic acid, and biotin along with high-carbohydrate and protein diet.	125
31	Pyruvate Dehydrogenase Deficiency	Infants and young children.	Dysmorphism with severe cerebral malformations.	PDHA1, PDHB, DLAT , PDHX / autosomal recessive	Cranial MRI and pyruvate and lactate levels in CSF and blood.	ketogenic diet, Diet supplemetation with thiamine, carnitine or lipoic acid. Phenylbutyrate or dichloroacetate	126-128

S.No.	Disorder	Age of Onset	Key Clinical Features	Gene implicated/ Inheritance Pattern	Diagnosis	Treatment	Reference
32	Short-Chain Acyl-CoA Dehydrogenase Deficiency (SCAD)	Infants and young children.	Dysmorphic facial features, metabolic acidosis, ketotic hypoglycemia, lethargy, seizures, hypotonia, dystonia, and myopathy.	ACADS/ Autosomal recessive	Assay for butyrylcarnitine concentrations in plasma and/or ethylmalonic acid concentrations in urine.	Avoidance of longer fasting. Use of carnitine and/or riboflavin supplementation.	129, 130
33	Short Chain Hydroxy Acyl-CoA Dehydrogenase Deficiency (SCHAD)	Infancy or early childhood	Hyperinsulinemic hypoglycemia with vomiting, lethargy and seizures.	HADH / Autosomal recessive	Measurement of body fluid and cultured cell 3-hydroxy fatty acids.	Diazoxide and chlorothiazide helps in controlling hyperinsulinism. Partial or total pancreatectomy.	131-133
34	Very Long-Chain Acyl-CoA Dehydrongenase Deficiency (VLCAD)	Neonatal, Early childhood and in Young adults.	Hypoketotic hypoglycaemia, liver disease, myoglobinuria, cardiac arrhythmias and cardiomyopathy.	ACADVL and DLG4/ Autosomal recessive.	Acylcarnitine profile in blood and plasma. VLCAD deficiency using immunohistochemistry.	Dietary treatment is primary. High carbohydrate-low fat diet. Supplementation with carnitine and/ or riboflavin. Treatment with bezafibrate offers benefit in myopathic patients.	134, 135

pyruvate quantification in blood and CSF. An increased level of lactic acid is one of the important characteristics of mitochondrial disorders.[43-46] Lactate to pyruvate ratio reflects cytoplasmic status.[47] Elevation of tyrosine, alanine and/or phenylalanine indicates hepato-cerebral form of mtDNA depletion.[48] Measurement of these amino acids in CSF and blood may be desirable. Dicarboxylic aciduria reflects impairment of fatty acid beta oxidation, hence direct measurement of dicarboxylic acid in urine may be performed.[49] Detection of abnormal levels of carnitine and acyl-carnitine indicate fatty acid beta oxidation defects in cell, through tandem mass spectrometry.[50] Fibroblast growth factor-21, involved in lipid metabolism was found to be elevated in patients with mitochondrial skeletal muscle disorders.[51]

Proliferation of skeletal myofibers helps in using histological and histochemical tools in diagnosing the mitochondrial disorders.[52] Gomori trichrome staining can be used to visualize red granular deposits of mitochondria in the subsarcolemmal space of myofibre, which resembles ragged red fibers. These ragged red fibres are cytochrome oxidase (COX) deficient myofibres and succinate dehydrogenase (SDH) histochemistry can diagnose COX deficient fibres.[53] Normal COX fibres appear as brown and COX deficient fibres stain poorly. But on repeated staining these fibres give dark blue colour. Hematoxylene-eosine staining shows scattered abnormal vacuolated fibres with clear rim.[53] Immunohistochemistry uses antibodies raised against specific protein subunits of respiratory chain. Immunohistochemical staining of a muscle biopsy from Kearns Sayre syndrome showed normal levels of COX4 and reduced levels of COX2.[53] Anti DNA antibodies also can be used to detect abnormal mtDNA in muscle fibers. Electron microscopy can be used to detect abnormal ultrastructural changes such as, number, shape and size of mitochondria, absence of cristae and presence of paracrystalline inclusions in mitochondria.[54]

Various molecular biological techniques can be used to detect mutations, deletions, duplications and copy

number variations in mtDNA and/or nuclear DNA, to diagnose mitochondrial disorders. Common or known mutations can be detected using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).[55,56] Rare and uncommon mutations can be detected using direct sequencing or denaturing HPLC.[57] Heteroplasmic mutations and copy number variations can be detected using quantitative real time PCR.[58] The deletions and duplications in mtDNA can be assessed by using southern blotting or PCR based strategies.[59] Advancement and automation in the sequencing technology in the form of next generation sequencing, has replaced multiple techniques and made the analysis of entire nuclear genome as well as mitochondrial genome possible.[60-63] Other assays include measurement of electron transport chain enzyme complex activities based on the absorbance change of the substrate, either NADH or cytochrome c.[64] Measurement of oxygen consumption or oxidative ATP synthesis rates in live cells or isolated mitochondria reflects the integrity of inner mitochondrial membrane and efficiency of oxidative phosphorylation. The respiratory chain complexes can be separated on Blue native PAGE and detected using immunoblotting with commercially available antibodies.[65] Measurement of coenzyme Q levels in plasma, WBCs and other tissues can be performed by HPLC to know the oxidative stress caused by mitochondrial disorders.[66] Based on the patients' clinical, histological, enzymological, functional, molecular and metabolic evaluations, consensus general diagnostic criteria were made.[67] According to these criteria, for the definite diagnosis of respiratory chain disorders in adults and children, one should follow two major or one major and two minor criteria for definite diagnosis.

CLINICAL SPECTRUM

As described elsewhere in this review, mitochondrial diseases caused by mutations in mtDNA or nuclear encoded mitochondrial genes that are involved in a variety of aspects of energy metabolism and oxydative phosphorylation. Mutations in set of genes involved in aerobic respiration and maintenance of mtDNA cause depletion of mtDNA content in the skeletal muscle or liver cells. Mictochondrial depletion syndrome can present clinically as a mitochondrial myopathy, encephalopathy or encephalohepatopathy. Mitochondrial disorders affect many organ systems that have more mitochondria, such as brain, nerves, muscles, kidney, heart, liver, eyes and ear (**Figure 1**). Mitochondrial disorder affecting brain leads to developmental delays and mental retardation.[68] Mitochondrial dysfunction contributes to the development of muscle disorders, including muscle wasting, atrophy and degeneration.[69,70] Involvement of

Table 2: Summary of Investigation Tools Used to Diagnose Mitochondrial Disorders.

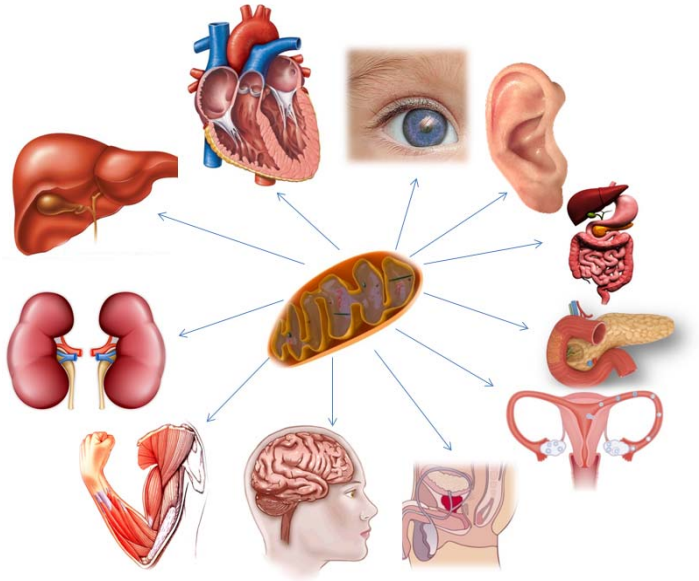
Minimally Invasive Investigations	Invasive Investigations	Molecular Biology Techniques
Blood and Urine Chemistry: • Lactate and pyruvate quantification in blood and CSF • Elevation of tyrosine, alanine and/or phenylalanine in blood and CSF • Dicarboxylic acid, ethylmalonic acid in urine • Carnitine and acyl-carnitine, butyryl-carnitine through tandem mass spectrometry • Coenzyme Q levels in plasma, WBCs and other tissues through HPLC • Plasma thymidine, deoxyuridine • Thymidine phosphorylase enzyme quantification in leukocytes **Imaging:** • Brain and spinal cord MRI and MRS	**Histological and Microscopic Investigations:** • Gomori trichrome staining • Hematoxylene eosine staining • SDH histochemistry: to detact COX deficient fibres • Immunohistochemistry: • Antibodies against components of respiratory chain • Anti DNA antibodies • Electron Microscopy: to detect abnormal ultrastructural changes • Bone marrow biopsy • **Biochemical Assays:** • Measurement of electron transport chain enzyme complex activities • Separation of respiratory chain complexes through Blue native PAGE **Immunoblotting**	**Detection of Variations in mt and Nuclear DNA:** • Known mutations can be detected using PCR-RFLP • Uncommon mutations can be detected using direct sequencing or denaturing HPLC • Heteroplasmic mutations and copy number variations can be detected using real time PCR • Mt-DNA deletions and duplications can be assessed by southern blotting or PCR • Next generation sequencing

the peripheral nervous system in mitochondrial disorders contributes to the variability of their clinical expression.[71] Major renal manifestation of mitochondrial disorder is due to ATP involvement in driving the sodium-potassium-ATPase pump.[72,73] Renal biopsy of these shows non-specific abnormalities of the tubular epithelium with dilatation and obstruction by casts, dedifferentiation, or atrophy.[74] Mitochondrial cardiomyopathy is characterized by abnormal cardiac muscle structure, valvular disease with typical manifestations of hypertrophic and dilated cardiomyopathy, arrhythmias, left ventricular myocardial noncompaction.[75] As the liver is involved in a variety of critical biological functions, incapacitated mitochondrial bioenergetics triggers the pathogenesis of various hepatic diseases such as fatty liver disease, hepatitis, and liver cancer.[49,76] Blindness and deafness are two important problems respectively in eyes and ears.[77]

TREATMENT

There is no definite cure for the mitochondrial disorders, but treatment can delay the progression of disease or may provide a symptomatic relief.[78] Vitamins and cofactors are in use for treatment of mitochondrial disorders.[79] Thiamine, riboflavin, vitamin C & E and some enzymes such as lipoic acid and coenzyme Q10 are used to treat mitochondrial disorders.[78] B complex vitamins help in decarboxylation, fatty acid oxidation, acetyl-CoA synthesis and lipid metabolism.[80, 81] Vitamin C and Lipoic acid act as antioxidants and vitamin E and Coenzyme Q10 are free radical scavengers. Zinc picolinate is a superoxide dismutase and involved in tissue repair. Biotin is involved in carboxylation and lipid metabolism. However, clinical trials of the therapeutic utility of conventional antioxidants such as Vitamin E or Vitamin C have yielded disappointing results in patients with mitochondrial oxidative damage.[82, 83] Some drugs, that specifically block the lactic acid build up in body are also used for treating mitochondrial disorders.[84] Diet modulation by reducing carbohydrate in diet can be used to decrease the production of damaging free radicals and workload on mitochondria.[85] As the mitochondrial disorders involve defect in exceedingly fundamental level in cell function, no vitamin or cofactor therapy is curative. Further, therapy should be guided by a diet and nutrition specialist.[85] As there is no measurable evidence of improvement in function or disease status and vice versa, evaluating treatment outcomes is difficult. Hence the treating physician should remember that therapy without diagnosis leads to failure in establishing accurate diagnosis.

Figure 1: Mitochondrial Disorder Affects Multiple Systems of the Body



As mitochondrial disorders exhibit large amount of genetic and phenotypic heterogeneity, development of new drugs is literally a challenging task. However, different animal and cell models are being exploited for understanding and developing treatments. The cell models include yeast mitochondrial disease models and patient derived cell lines, while animal models include *Drosophila melanogaster, Cenorrhabditis elegans* and a plethora of mouse models. Further, delivering molecules to mitochondria is difficult due to relative inaccessibility of mitochondria matrix. Furthermore, extreme genetic and phenotypic heterogeneity pose difficulty in conducting clinical trials with adequately large groups of patients. Although there are no clinically relevant universally agreed and validated outcome measures, some pharmacological therapies targeting mitochondrial biogenesis (Bezafibrate, Resveratrol), mitochondrial membrane fluidity and plasticity and mtDNA replication machinery are under development.[50]

CONCLUSIONS

Mitochondrial disorders are extremely complex and involve multiple organ systems with multiple heterogeneous clinical presentations, including inflammation, metabolic syndrome, neuromuscular disorders and cancer. There is no single test to diagnose mitochondrial disorders due to their clinical variability and involvement of large number of nuclear and mitochondrial gene mutations. Although several attempts have been made to significantly modify the suspected

phenotype, mitochondrial dysfunction is now recognized as central in several medical conditions. Hence, novel therapeutic interventions that modify mitochondrial function *are currently* under development.

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