REVIEW

Diagnostic Challenges in Mitochondrial Disease

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It takes about 3000 genes to make a mitochondrion. Mitochondrial DNA encodes just 37 of these genes; the remaining genes are encoded in the cell nucleus and the resultant proteins are transported to the mitochondria. Only about 3% of the genes necessary to make a mitochondrion (100 of the 3000) are allocated for making ATP. More than 95% (2900 of 3000) are involved with other functions tied to the specialized duties of the differentiated cell in which it resides. Mitochondria contain the rate-limiting enzymes for pyrimidine biosynthesis (dihydroorotate dehydrogenase) and heme synthesis (d-amino levulinic acid synthetase) required to make hemoglobin. In the liver, mitochondria are specialized to detoxify ammonia in the urea cycle. Mitochondria are also required for cholesterol metabolism, for estrogen and testosterone synthesis, for neurotransmitter metabolism, and for free radical production and detoxification in addition to oxidizing the lipids, protein, and carbohydrates. Mitochondrial disease confirmation and establishment of a specific molecular diagnosis requires extensive clinical and laboratory evaluation. Dual genome origins of mitochondrial disease, multi-organ system manifestations, and an ever increasing spectrum of recognized phenotypes represent the main diagnostic challenges. To overcome these obstacles, compiling information from a variety of diagnostic laboratory modalities can often provide sufficient evidence to establish an etiology. These include blood and tissue histochemical and analyte measurements, neuroimaging, provocative testing, enzymatic assays of tissue samples and cultured cells, as well as DNA analysis.

Key Words : Mitochondrial disease, Genetics.

INTRODUCTION

The mitochondria, as we know is the energy producing organelle in all except the red blood cells of the human body. All the chemical reactions that take place in the body are energy dependent, either directly or indirectly, and so is the involvement of the mitochondrion in them. Hundreds of biochemical pathways consisting of many cycles and hundreds of enzymes are functioning in this ''power-house'' of the cell, as it is rightly called[1]. A mitochondrial disease is one that is caused basically due to defects at the molecular and genetic level which manifests as certain enzyme deficiencies or other related defects in the mitochondrial biochemical pathways. The mitochondrial DNA is mainly involved in these set of disorders. The two things that make a mitochondrial disease very unique are its inheritance pattern[2] and that fact that the mitochondrion is extremely critical to the various bodily functions. Weakness and much lesser energy production at the molecular level are generally accompanied by extreme variety of both, general and systemic clinical manifestations[3-6]. This makes it extremely difficult for a clinician to diagnose the exact pathology the patient is presenting with, as most of the symptoms may be suggestive of various other systemic disorders. Certain common symptoms which may be typical for a mitochondrial disorder are lethargy, headache, vomiting and dizziness and in many cases even muscle weakness and ataxia. The degrees of the severity of a mitochondrial disease vary greatly[7-18]. Most of mitochondrial diseases are seen in pediatrics age group. This is because, any mitochondrial defect gives rise to the manifestations in the first few months or in some cases, even first few days of life. Some adult mitochondrial diseases are also found, and may be due to certain secondary complications, which is discussed in the following part of the article.

CLASSIFICATION OF MITOCHONDRIAL DISEASES

Following are the various major types of mitochondrial disorders[1,19,20]

1. Mitochondrial Encephalomyelopathy Lactic Acidosis and Stroke like syndrome (MELAS)[3,7]
2. Myoclonic epilepsy and ragged red fibres (MERRF)[21]
3. Kearns-Sayre Syndrome (KSS)[22]
4. Chronic Progressive External Ophthalmoplegia (CPEO)[23]
5. Diabetes mellitus and Deafness (DAD)[17]
6. Leber's Hereditary Optic Neuropathy[3,24]
7. Leigh Syndrome[25,26]
8. Myoneurogenic gastrointestinal encephalopathy (MNGIE)[27]
9. Defects in amino acid metabolism[1]
10. Urea cycle disorders[1]
11. Disorders of mitochondrial fatty acid β-oxidation[1]
12. Lipidoses lysosomal storage disease[1]
13. Disorders of carbohydrate metabolism[1]
14. Disorders of glycoprotein degradation and structure
15. Congenital disorders of glycoprotein (CDGs)
16. Disorders of the mitochondrial respiratory chain (ETC disorders)[1,18,28]
17. Wolfram Syndrome[11]

Some of the above mentioned disorders like the urea cycle defects, the disorders of fatty acid β-oxidation or the carbohydrate metabolism disorders have further classifications based on various enzymes that are present in the biochemical pathways affected in the respective disorders.

A defect in the mitochondria gives rise to or further worsens disease like the Parkinson's disease[29], Alzheimer's disease[30,31], Type 2 diabetes[12,15], atherosclerotic disease and some cancers. For example, renal mitochondrial mutation and protein damage has been in observed in type 2 diabetes. Thus, mitochondrial damage also plays a role in diseases other than those who have their cause at the mitochondrial or nuclear genetic level.

CLINICAL MANIFESTATIONS AND BIOCHEMICAL FINDINGS

A large number of general and systemic symptoms are observed in most of the mitochondrial diseases[19]. In many mitochondrial diseases, the neuronal and muscular system is extensively affected[7]:

- Ataxia[9]

- Myopathy[5,32,]
- Encephalomyopathy[4,33]
- Deafness[11]
- Peripheral neuropathy[33]
- Seizures and convulsions[1]
- Muscle weakness[1]
- Visual impairment[23]

Certain general clinical manifestations like vomiting, lethargy, etc. are present in majority of the disorders with mitochondrial dysfunction. Cardiomyopathy and stroke like condition[5] indicates the involvement of the cardiovascular system[28,34]. Also, epilepsy is observed in many of the cases along with a risk of status epilepticus[8]. Along with these, the renal[16] and hepatic systems are too involved in majority of the mitochondrial diseases in some way or the other. In fact, hepatopathies[35] are observed in many infants diagnosed with mitochondrial disease. Some mitochondrial disorders may present with associated hematological disorders like aplastic, megaloblastic, or sideroblastic anemia, leukopenia, neutropenia, thrombocytopenia, or pancytopenia[19]. Besides this, the psychiatric presentations in the cases of mitochondrial disorders included mood disorder, cognitive deterioration, psychosis, and anxiety[32, 36-39].Thus, we can see that multi-organ pathologies and involvement of multiple, almost all systems of the body maybe indicative of a mitochondrial disorder.

The mitochondrial diseases, always present with some or the other peculiar biochemical findings[6,20,34,35,40] along with many common findings. For example, in the urea cycle disorders, hyperammonemia[1] is a characteristic feature. Be it any enzyme deficiency in the urea cycle, hyperammonemia will be present which can be used for a differential diagnosis for the urea cycle defects. Similarly, there will be hypoglycemia and increased levels or some enzyme specific dicarboxylic acids in the urine in most of the fatty acid β-oxidation disorders[1]. Hence, it is extremely important to carry out disease specific biochemical laboratory tests [40] to reach a definitive diagnosis.

GENETICS AND OCCURRENCE OF MITOCHONDRIAL DISEASE

Mitochondrial disease can be caused due to mutations in both nuclear and mitochondrial DNA. However, the mutations in the mitochondrial DNA are more frequently observed. Inheritance pattern in this case is purely maternal, fathers cannot pass on the defective mitochondrial genes to the offspring[2,38]. Hundreds of mitochondrial mutations that cause a disease have been identified[41]. These are present on various loci of the circular mitochondrial DNA. For example, the Leber's Hereditary Optic Neuropathy (LHON)[24] is due to a mutation in the gene ND1 on the mitochondrial DNA[1]. Neuronal oxidative phosphorylation (OXPHOS) deficiency has been associated with a variety of neurodegenerative diseases, including Parkinson's disease and Huntington's disease[10].

The occurrence of mitochondrial disease is 1in 4,000 in the United States of America. Indian statistics, as of now for mitochondrial disease are not available. Some diseases also occur in the adult age group, though the occurrence is less. However, as mentioned above, a pathology in the mitochondria may be a cause for Alzheimer's disease[31,42], Parkinson's disease[29], Type 2 Diabetes[13,15], etc. for example, renal mitochondrial damage is seen in type 2 diabetes[16].

DIAGNOSIS OF MITOCHONDRIAL DISEASE

Neuroimaging and neuropsychological studies are indicated in individuals with suspected central nervous system (CNS) disease. Electroencephalography (EEG) is indicated in individuals with suspected encephalopathy or seizures. Peripheral neuropsychological studies are indicated in individuals with limb weakness, sensory symptoms, or areflexia. Magnetic resonance spectroscopy and exercise testing (with measurement of blood lactate concentration) may be used to detect evidence of abnormal mitochondrial function noninvasively. An elevated concentration of fasting blood glucose may indicate diabetes mellitus. Cardiac electrocardiography (EKG) and echocardiography (ECHO) may indicate cardiac involvement. Biochemical laboratory finding is an important diagnostic criterion in a mitochondrial disease[1]. The following parameters are frequently checked for in suspected mitochondria disease cases:

- Plasma ammonia levels

- Lactate
- Pyruvate
- Some disease specific amino acids
- Blood sugars and lipids

Lactate/pyruvate measurement of blood lactate concentration is indicated in individuals with features of a myopathy or CNS disease. A fasting blood lactate concentration > 3 mm/l supports a diagnosis of mitochondrial disease. Measurement of cerebrospinal fluid (CSF) lactate concentration is indicated in individuals with suspected CNS disease. A fasting CSF lactate concentration > 1.5 mm/l supports a diagnosis of mitochondrial disease. "Modified Walker Criteria"[1] is a diagnostic criteria applied to children referred for evaluation of mitochondrial disease in the US. It has certain parameters which are checked for in every case:

- Clinical
- Histology
- Enzymology
- Functional
- Molecular
- Metabolic

Molecular genetic testing can be carried out on genomic DNA extracted from blood (suspected nuclear DNA mutations and some mtDNA mutations), or on genomic DNA extracted from skeletal muscle (suspected mtDNA mutations). Southern blot is a molecular genetic testing technique used to detect a pathogenic mtDNA rearrangement. The deletion or duplication breakpoint may be mapped by mtDNA sequencing. The molecular diagnosis of mitochondrial diseases is generally based on Sanger sequencing and PCR-RFLP[38] .Muscle biopsies[43] are also done for the diagnosis in certain cases. Basically, the morphology of the mitochondria is checked for in the biopsies which can be taken from the muscle or any other tissue which is suspected to have a mitochondrial pathology. Also, some techniques like gas chromatography-mass spectroscopy[44] are being used for diagnostic purposes. Also, a brain MRI is used for detecting lactate peaks[45]. Thus, we can say that advancements have taken place in the diagnostics of the mitochondrial diseases in the past decade. MtSNP score is another diagnostic tool developed for the identification of the mitochondrial mutation[14].

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