Review Article

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Dentistry Section

Mitochondria: Physiology & Pathology- An Insight into the Less Known Entity

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ABSTRACT

Mitochondria are the small cellular organelle, considered to be the powerhouse or batteries of the cell. Unlike other organelles, they are unique due to the special characteristics they possess. They contain their own genetic material and are exclusively maternally inherited. Earlier it was considered as just an energy producing organelle, but the research studies have explored the other roles of mitochondria in normal physiology and pathology as well. It can produce its own proteins through the genetic material possessed in it. On contrary the dysfunctioning of the organelle and the accumulating genetic mutations in its DNA can cause numerous disorders. Its unique characteristics make it well suited for forensic analysis. This article gives an overview of the normal physiology of the mitochondria and associated mitochondrial disorders.

Keywords: Mitochondria, Energy, mtDNA, Heteroplasmy, Mitochondrial disease

INTRODUCTION

Mitochondria are the ovoid or elongated thread like membrane bound organelles of great metabolic significance and form the principle source of chemical energy [1,2]. They are approximately 0.5um – 1um in diameter and 5um – 10um in length, with the exception of cardiac muscle mitochondria which is comparatively larger in size [2,3]. The number of mitochondria in a cell differs in relation to their energy requirements, and they are situated close to the parts of cell that shows highest energy requirements [1]. They are present in all human cells except mature erythrocytes [4,5]. Mitochondria are self replicating and they increase in number by division throughout interphase, and their division is not synchronized with the cell cycle [2,3].

Mitochondria are thought to have originated billions of years ago as primitive bacteria. They have developed from the order of proteobacteria, the rickettsiales as endosymbionts. Over thousands of generations, some of the genetic information from these bacteria have migrated into what has become human cell nuclei, while the mitochondria now exist separately within the cell cytoplasm, retaining their own independently-replicating DNA [1,6].

Structure

Mitochondria display a variety of shapes, including spheres, rods, elongated filaments and even coiled structures. They are surrounded by double membrane and structurally consist of outer membrane, inner membrane, inter membranous space and mitochondrial matrix. Each of these components plays distinct functional roles, with the matrix and inner membrane representing the major working compartments. Each mitochondrial membrane is separated by a narrow intermembranous space. The inner membrane surrounds the mitochondrial matrix and the outer membrane is in close contact with the cytoplasm [2,7,8].

Outer membrane contains many voltage dependent anion channels, called mitochondrial porins, which are permeable to uncharged molecules upto 5000 daltons. This membrane is sometimes attached to other organelles such as microtubules and it also possess receptors for proteins and polypeptides that translocate into the intermembrane space [3].

Intermembrane space is the narrow space that separates the inner and outer mitochondrial membranes. As the outer membrane is freely permeable to many substances, small molecules, ions and metabolites can enter the intermembrane space but cannot penetrate the inner membrane. The composition of this space therefore is similar to that of the cytoplasm. It provides a closed compartment into which protons can be pumped by the inner mitochondrial membrane to maintain the gradient necessary for the transport of various ions and molecules. It also contains specific enzymes that use the ATP generated in the inner membrane [3,8].

Inner membrane is deeply folded into cristae whose number and extension is directly related to the energy requirement of the cell. They also show tissue specific variations reflecting

their chemistry. Inner membrane is selectively permeable, being virtually impermeable to ions, but can actively transport metabolites from and into the central matrix. The presence of cardiolipin, an unusual phospholipid may contribute to this relative impermeability [3,6,9]. The membrane forming the cristae contains proteins that aid in performing oxidation reactions, synthesis of ATP and regulating the transport of metabolites into and out of the matrix. Enzymes responsible for oxidative metabolism are embedded in the inner mitochondrial membrane and form a characteristic repeating unit. Each unit is made up of a basepiece, a stalk and spherical headpiece. Base piece contains enzymes for electron transport chain. Stalk and spherical headpiece contains ATP and other enzymes concerned with ATP synthesis [1,2]. Mitochondrial Permeability Transition (mPT) are the high conductance channels of inner mitochondrial membrane formed due to increased cytosolic calcium, intracellular oxidative stress and lipid breakdown [3,9].

Mitochondrial matrix is an aqueous environment containing filaments of mitochondrial DNA (mtDNA), mitochondrial ribosomes with rRNA, matrix granules and multitude of enzymes responsible for the central reactions of oxidative metabolism. Matrix granules are the cation accumulations primarily consisting of calcium phosphate that helps to maintain a characteristically low level of calcium in the cytoplasm. The presence of granules testifies to the marked capacity of mitochondria for accumulating calcium ions in the matrix. Apart from storing calcium the granules can also store other divalent and trivalent cations. These granules increase in number and size when the concentrations of these cations increase in the cytoplasm. Mitochondria can accumulate cations against a concentration gradient. Thus, in addition to ATP production, mitochondria also regulates the concentration of certain ions of the cytoplasmic matrix, a role they share with smooth endoplasmic reticulum [3,9].

Mitochondrial DNA

Mitochondria's genetic system, the mtDNA was discovered in 1963. In 1981, the mitochondrial genome became the first complete sequence of a human genome to be published [1]. It differs from nuclear DNA (nDNA) in many aspects [Table/Fig-1]. Mitochondrial genome contains a ~16.5 kb circular DNA, small sized rRNA and about 3000 genes [1,6,10,11,12] [Table/Fig-2]. Only about 3% of the genes (100 of the 3000) are allocated for making ATP and more than 95% (2900 of 3000) are involved with other functions such as pyrimidine, heme, estrogen and testosterone synthesis, cholesterol metabolism, free radical production and detoxification [8]. Mitochondrial proteins are originated mainly from two sources. While most mitochondrial proteins are translated on free cytosolic ribosomes, few other proteins are synthesized by their own genomes. Their membrane lipids are synthesized by endoplasmic reticulum [9].

The mtDNA is exclusively maternally inherited [1,11]. Sperm contains mitochondria, which are used to help power their movement, but immediately after fertilisation these paternal mitochondria degenerate as the male pro nucleus forms in the fertilised egg. Only one study has found paternal mitochondria to persist naturally after fertilisation (in muscle tissue only) [11].

Cambridge Sequence is the standard sequence to which all the human mitochondrial DNA are compared. It was sequenced from several different human mtDNAs by a Medical Research Council Laboratory based at Cambridge, UK. Variations in the mtDNA genome, from the Cambridge sequence is termed polymorphism. When the mtDNA of any single person is sequenced, a number of variations from the Cambridge Sequence are noted. In vast majorities of these, the differences are simply polymorphisms and are not clinically significant. Control Region or D-loop is highly polymorphic and hence is used for forensic purposes in providing a " DNA finger print "of suspects in criminal investigations [8].

Mitochondria and its genome exhibit several unique characteristics such as multiple genome copies, heteroplasmy, variable expressivity, mitotic segregation and the threshold effect, that affect the inheritance pattern and phenotype of the mitochondrial diseases [5,6,7,8,11]. Heteroplasmy and variable expressivity is the presence of a mixture of more than one type of mtDNA and only a fraction of molecules carry the mutation. Female primordial germ cell contains mixture of normal and mutated mtDNA. The fraction of mutated mtDNA molecules within a cell is determined by a combination of random chance & selection at the cellular level during embryonic development. Heteroplasmy refers to this coexistence of wildtype (naturally occurring, non mutant) and mutant mtDNA within the same cell (intracellular heteroplasmy) and between different cells (intercellular heteroplasmy). Threshold expression is a state when more than half of the mtDNA copies are defective, causing mitochondrial disease to become more pronounced and unavoidable. The trait of mitochondrial diseases is that identical mtDNA mutations may not produce identical disease [5]. The clinical phenotype of patients with mitochondria related disorder may not remain constant and it may change as they grow older. This is because the mitochondria expresses the phenomenon of mitotic segregation, which means that the propotion of mutant mtDNAs in daughter cells may shift and the phenotype may change accordingly [5,6].

Mitochondria have been used for studying human evolution from maternal perspective due to their maternal inheritance. They have been valuable tools in knowing the origin of human species, migrations and colonization of new regions of the

world [11]. mtDNA acts as good source of evidence in forensic investigations. It can be used even in cases when the biological evidence may be degraded or is in small quantity or in cases where evidence consists only of hairs, bones and teeth. Since it is available in multiple copies, it allows detection of much lower amount of the sequence of interest, especially that from fossil material [11]. Mitochondria have defensive property as well. They exhibit antiviral immune responses by activating retinoic acid-inducible gene I (RIG-I)-like receptors signal transduction pathway. They perform this in interconnection with other organelles, such as the endoplasmic reticulum (ER) and peroxisomes [13].

Mitochondrial Diseases

Mitochondrial diseases are the result of either inherited or spontaneous mutations in mtDNA or nDNA. It leads to altered function of the proteins or RNA molecules that normally reside in mitochondria. It was 1st diagnosed in 1962 and studies have shown that the overall prevalence of mitochondrial diseases is about 5/100000 accounting for about 15% of the total. It is estimated that, about 4,000 children develop mitochondrial disease by the age of 10 years and 1000 to 4,000 children per year are born with a type of mitochondrial disease [4,5,6,8].

mtDNA is easily susceptible for damage by reactive oxygen species, due to the reason that it lacks protective histone backbone and complex DNA repair mechanism associated with nDNA [14]. Like nDNA, it can also be altered by mutations, that are frequently deleterious to the organelle. Mutation rate is over 10 times that of nuclear DNA [5,11]. Because of the high gene density of the mitochondrial genome, mutations in mitochondrial DNA (mtDNA) have a high likelihood of affecting the expression or coding sequence of mitochondrial genes. This susceptibility to deleterious mutations is offset to an extent by the presence of multiple copies of the mitochondrial genome in every cell [8]. Pathogenic mtDNA mutations may be point mutations, large-scale rearrangements such as single deletions, duplications or multiple deletions or mtDNA depletion [5]. In general, the higher the mutational load of mtDNA within the tissue or cell, the greater the level of mitochondrial dysfunction [5].

	Nuclear DNA (nDNA)	Mitochondrial DNA (mtDNA)
• Location	• Found in nucleus of the cell	• Found in mitochondria of the cell
• Number	• 2 sets of 23 chromosomes	• Each mitochondria has several copies of the single mtDNA molecule
• Shape	• Double helix	• Circular
• Structure	• Bounded by a nuclear envelope	• Doesn't have a nuclear envelope
• Chromatin	• DNA packed into chromatin	• DNA is not packed into chromatin
• Inheritance pattern	• Both Maternal and paternal	• Maternal only
• Discrimination	• Can discriminate between individuals of the same maternal lineage	• Cannot discriminate between individuals of the same maternal lineage
• Mutation rate	• Susceptible for mutation	• Susceptible for mutation and is 10 times higher than nDNA
• Forensic Evidence	• Good aid in forensic investigations	• Can be used even in cases where the sample is very small and degraded

[Table/Fig-1]: Differentiating features between nuclear DNA (nDNA) and mitochondrial DNA (mtDNA)

• 16,569 base pairs
• Encodes - 37 genes
• Codes 13 protein subunits – mainly responsible for certain key components of oxidative phosphorylation pathway
• Genes for 12s and 16s rRNA
• Genes for 22 tRNA
• D loop containing DNA replication and transcriptional promoter sequence
• Maternal inheritance
• Multiple copies
• No effective DNA repair system
• Subjective to mutational changes

[Table/Fig-2]: Characteristics of mtDNA

Mitochondrial diseases show different modes of inheritance [1,5,8,11]. Unlike nDNA, where inherited mutations are almost always present in the same number in every cell of the body, the abundance of mtDNA mutations can vary dramaticaly from cell to cell, and even from tissue to tissue. As all the mtDNA are contributed from the oocyte, the genetic variants are generally passed only through the maternal line. Men will be affected by mitochondrial disorders when there is sufficiently high proportion of mutated mtDNA and they do not pass their mitochondria on to their children. There is no evidence

of father-to-child transmission of an inherited mitochondrial disorder [1]. If nDNA gene trait is recessive, often other family members are not affected and there is a 25 percent chance of the trait occurring in other siblings. If this gene trait is dominant, the disease often occurs in other family members and there is a 50 percent chance of the trait occurring in other siblings. If the inheritance is through mtDNA there is a 100 percent chance of the trait occurring in other siblings, since all mitochondria are inherited from the mother, although symptoms might be either more or less severe. Combination of mtDNA and nDNA defects, relationship between nDNA and mtDNA and their correlation in mitochondrial formation is unknown. Medicines or other toxic substances can also trigger mitochondrial disease [1,3,8].

Symptoms of Mitochondrial Disease

Mitochondrial disease exhibit varied clinical patterns. They can affect either a single organ or involve multiple organ systems. Symptoms may not appear for years after birth, even when the condition is inherited. They may be totally absent in silent carriers. It can be a debilitating, life threatening condition in its severest form and such forms are more frequent in children [5]. Adult patients often show chronic multisystem clinical manifestations. They cause the most damage to cells of the brain, heart, liver, skeletal muscles, kidney and the endocrine and respiratory systems [Table/Fig-3]. Symptoms may be very common ones such headache, malaise, fever, seizure, weakness, muscle pain etc. which occur in many other diseases as well, thus making the diagnosis difficult. On the other hand it may show unusual presentations of the usual diseases [4,5,8]. Hence, it is suggested that when a common disease manifests with features that sets it apart or it involves more than three organ systems, mitochondrial disorders need to be thought of [8]. The disease and its varied pattern mainly depends on the tissue distribution of mtDNA mutations, the threshold effects of the tissue at the time, environmental stress and underlying nuclear genetic factors [5]. The various mitochondrial diseases have been listed in [Table/Fig-4].

• Poor growth, Weakness
• Muscle weakness
• Dysphagia
• Dysarthria and Poor coordination
• Visual and Hearing problems
• Reduced mental functions
• Disease of organs (heart, liver, kidney)
• Neurological involvement – migraine like headache, seizures, dementia, ataxia
• Respiratory problems
• Gastro-intestinal disorders and swallowing difficulties
• Diabetes milletus, Lactic acidosis
• Susceptibility to infection
• Endocrine abnormalities – Rarely hypoparathyroidism

[Table/Fig-3]: Varying clinical manifestations of mitochondrial diseases

• Leber's hereditary optic neuropathy (LHON)
• Myoclonic epilepsy-ragged red fibers syndrome (MERRF)
• Myopathy-encephalopathy-lactoacidosis stroke syndrome(MELAS)
• Chronic progressive external ophthalmoplegia (CPEO)
• Neurogenic ataxia retinitis pigmentosa syndrome (NARP)
• Mitochondrial myopathy, neuropathy, gastrointestinal encephalopathy syndrome (MNGIE)
• Sensory ataxic neuropathy, dysarthria and ophthalmoparesis (SANDO)
• Oncocytoma, Burkitt's lymphoma (BCL2), Adrenocortical & Renal tumors
• Type II diabetes mellitus
• Anemias, Vitamin deficiencies, Mineral toxicity
• Multiple sclerosis, Systemic lupus erythematosis
• Rheumatoid arthritis, Thyrotoxicosis
• Atherosclerotic heart disease, Congestive heart failure
• Parkinson disease, Alzheimer's disease, Dementia
• Cancer metastasis
• Aging

[Table/Fig-4]: List of few mitochondrial diseases

Mitochondria are the main targets for ROS (reactive oxygen species), which are produced by themselves. ROS produces oxidation of mtDNA residues leading to deletions and rearrangements. Lipids in the mitochondrial and cellular membrane are peroxidized, becoming stiff and leaky. Proteins may be damaged and partially unfolded by oxidation. Many of these mtDNA modifications are mutagenic and are thought to contribute to cancer, ageing and neurodegenerative diseases [8,12].

Mitochondria induce apoptosis by increasing its pores & permeability or by mitochondrial swelling. Nitric oxide induces apoptosis by dissipating the membrane potential of mitochondria. Mitochondria induces apoptosis mainly by acting through SMACs (second mitochondria-derived activator of caspases), inhibitor of apotosis proteins (IAPs) - Cytochrome C – Apaf-1 – caspase [9,12]. The progressive accumulation of mutations in mtDNA has been suggested to contribute to the process of ageing. Usually the events such as free radical-induced oxidative damage or nucleotide mis-incorporation

during replication causes mtDNA mutations. Such extensive mutations result in electron transport alterations, further resulting in increased activation of apoptosis and thus the mitochondrial dysfunction [6,8,10].

mtDNA mutations have been reported in many type of cancers [3,12,14]. These mutations may be either base substitutions, occuring in protein-coding mitochondrial genes or the D-loop region which is the most frequent site of mutation in mtDNA [12]. Cancers of breast, lungs, stomach, thyroid, colon, rectum, bladder, prostate, ovarian, including head and neck have been associated with mtDNA mutations. These cancer related mitochondrial defects may be due to abnormal activity of respiratory chain subunits, decreased oxidation of NADH-linked substrates, altered expressions of mtDNA or mutations in mtDNA. mtDNA mutations causing respiratory deficiencies can further contribute to carcinogenesis by releasing abnormally high levels of reactive oxygen species into the cytosol, thus exposing the cellular organelles and the nucleus into cytotoxic compounds. mtDNA mutations have also been noted in oncocytes in Warthin's tumor. The oncocytes in the tumor may show mtDNA mutations or there may be presence of morphologically abnormal and respiratory deficient mitochondria [3,14]. Studies have shown that the mtDNA mutations can be used as biomarkers for early detection of cancer. Anticancer agents such as mitocans, act through the mitochondria, by targeting mitochondrial metabolism and apoptotic processes. Hence, mitochondria may become cellular targets for future cancer therapy [3].

Due to the varied clinical manifestations of these diseases, there are no absolute diagnostic criterias and classification for these diseases [4,8]. Most screening tests are neither specific nor sensitive and can lead to false-positive and false-negative diagnoses. Standardized treatment protocol does not exist and there is no cure for mitochondrial disorders [1,5,8]. Presently, dietary, vitamin and co-factor therapy has been tried and practiced for the treatment [8]. In general, the diagnosis and management of mitochondrial diseases involve a four-step process, confirming the diagnosis, assessing the illness, treating the symptoms and implementing preventive strategies [5].

CONCLUSION

The role of mitochondria as the chemical powerplant is well established fact since years. However, the recent studies have shed light on the numerous diseases associated with the dysfunction of mitochondria and the mutations related with its genome. The great hindrance in the diagnosis of these diseases is their usual symptoms or the unusual presentations. Due to the lack of the diagnostic criteria's and confirmatory diagnosis of these diseases, the management still remains uncertain. The thought of mitochondrial disorder in a health set up of developing countries like India is still at grass root level. Further research into the subject might improve our understanding of the subject and aid in the better management of such diseases.

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