

# Mitochondrial Diabetes: Differential Diagnostic Features and its Possible Management

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**Abstract:** Mitochondrial Diabetes (mtDB) is a rare form of diabetes and caused due to defects in mitochondrial DNA (mtDNA). The most reported genetic defect for this form is A3243G mutation in the mitochondrial DNA-encoded tRNA<sup>Leu(UUR)</sup> gene. It causes an adult onset form of diabetes that can be misdiagnosed as either Type 1 or Type 2 depending on the age of onset. As per literature, this form of diabetes is rare in occurrence and has various overlapping clinical features with other form of diabetes too; probably because of it only most of the people are not aware about this very form of diabetes. However it can be clinically diagnosed based on cumulative comparison of several parameters like age of onset, additional clinical features etc. Appropriate diagnosis is crucial for the management of mtDB in early stage itself. The proposed review discusses about the concept of mtDB, its distinguishing clinical features, and genetic causes as well the possible management of the phenotype.

## INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with defective carbohydrate and fat metabolism. These defects are mostly due to impaired insulin secretion from pancreatic beta cells and/or insulin resistance to the target cells like skeletal muscles, adipose tissues, and liver etc.

Vast majority of cases of diabetes fall under two major categories of diabetes mellitus as defined by WHO in 1980. <sup>1</sup> The first category is Type1 diabetes (T1D) or insulin dependent diabetes mellitus (IDDM) where the cause of hyperglycemia arises as absolute deficiency of insulin secretion. Occurrence of this type of diabetes among population is identified by the evidence of autoimmune pathologic process in pancreatic beta cells as a cause of low insulin secretion. The second category of diabetes named as Type 2 diabetes (T2D) or Non Insulin Dependent Diabetes Mellitus (NIDDM) is the most prevalent form that develops due to the combined action of both insulin resistance and insufficient insulin secretion. Along with these common forms, the other two types of diabetes such as Gestational Diabetes and Maturity Onset Diabetes of Young (MODY) are also reported to exist. Gestational diabetes is the state of glucose intolerance that develops during pregnancy and mostly disappears after delivery. On the other hand MODY is genetically inherited disorder which develops before the age of 25 yrs with the signs of hyperglycemia and thinning of patients.

The pathogenesis of Diabetes mellitus is very much under the control of mitochondrial activity. Production, release and activity of insulin are entirely dependent on mitochondrial function *i.e.* ATP production.<sup>2,3</sup> The dys-regulation of mitochondrial activity slows down the rate of insulin exocytosis from beta cell as well as development of insulin resistance in target tissues.<sup>2,4,5,6,7</sup> Mitochondrial dysfunctioning is majorly governed by increased oxidative stress that not only, restricts the energy production which ultimately impaired the insulin secretion but also; develop the state of insulin resistance. Mitochondrial genome is highly susceptible to oxidative stress as i) It is naked of histone proteins like in case of nuclear genome. ii) mtDNA lacks an adequate repair system hence, unable to cope with any variation incorporated during replication and/or damages caused due to mutagens. iii) mtDNA is located near inner mitochondrial membrane which is a hot spot for ROS generation and hence, is more susceptible for oxidative damage caused due to ROS. iv) The entire mitochondrial genome consists of coding sequences hence, all the variations are expressed.<sup>4</sup> Thus due to very high mutagenic susceptibility of mtDNA, it leads to altered expression of mitochondrial genes followed by reduced oxidative capacity and then compromised mitochondrial activity. Amongst large no. of mutations of mtDNA, some mutations are known to be associated with low insulin secretion *i.e.* diabetic phenotype. As per reports published in past decade, it turns out that there is another form of diabetes, where the specific mutations in mitochondrial genome that is A3243G

(tRNA<sup>Leu(UUR)</sup>) develop in to the state of hyperglycemia. This mutation in mt genome causes an adult onset form of diabetes and the average age of onset is 38 years. This mutation has 100% penetrance and hence those who have this gene will eventually become diabetic, with a form of diabetes characterized by failure to secrete insulin. As this type of diabetes is caused due to mutation in mt genome, hence it is categorized as mtDB.

## MITOCHONDRIAL DIABETES & ITS GENETICS

mtDB is the state of mitochondrial dysfunctioning caused due to mutations in mitochondrial genome. The mutations in mtDNA are characterized by attenuated cytosolic ADP/ATP levels, impaired insulin exocytosis from beta cells and development of additional clinical disorders like MELAS, cardiomyopathy, retinopathy etc.<sup>8</sup>

Mitochondrial diabetes is maternally inherited disorder as mtDNA is inherited maternally with vertical non-Mendelian pattern. It progresses at around the age of 38 years with no symptoms of obesity. The pathogenesis of mtDB involves age dependent mitochondrial dysfunctioning factors like increased ROS production, reduced expression of antioxidant enzymes and reduced mtDNA copy number which all result in respiratory depression along with reduced insulin secretion with higher level of glycemia.<sup>8</sup> These patients develop the disease at an early age and since hyperglycemia may be severe in these patients, diabetic complications are frequently observed.

As far as, mitochondrial genome is concerned, tRNA genes make up only 10% of its genome, but the mutations in these regions were reported to be "hot-spots" region for mutation-based mitochondrial pathogenesis.<sup>8</sup> Several tRNA mutations like A3243G, A8344G and many more affect the mitochondrial translation process and attenuate cytosolic ADP/ATP levels which further results in lower insulin secretion.

Among these, A3243G mutation is the most common mutation and even listed as a most important cause for mitochondrial diabetes. It is a point mutation at nucleotide 3243, associated with decoding defect of UUG (or UUA) codons and amino acid mis-incorporation caused due to mitochondrial translational malfunctioning. The pathogenic mechanism of A3243G mutation is associated with poor aminoacylation and reduced stability or lack of wobble-base U hypermodification of mutant tRNA molecules. The loss of post-transcriptional taurine modification at anticodon wobble position (required to restrict decoding to leucine UUR codons) leads to mitochondrial translational malfunctioning which, further results in impaired oxidative phosphorylation (OXPHOS) system and hence, impaired beta cells mediated insulin release. A3243G mutation not only, results in impaired translation but also, diminishes 16S rRNA transcription termination and alters the processing of primary transcript too.<sup>8</sup>

The result of the mutational studies indicates that the pathogenic A3243G mutation has two fold effect that it disrupts the structure and function of mt tRNA<sup>Leu(UUR)</sup> by destabilizing the native fold and promoting dimerization. The deleterious effect of this mutation attributes to decrease in protein synthesis which ultimately diminishes the respiratory load of -beta cells and hence the rate of insulin secretion.<sup>9</sup>

The diabetogenic character of A3243G mutation is stronger as compared with

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other mitochondrial mutations because A3243G mutation incorporates the deregulation of complex interaction between mitochondrial and nuclear genomes. Along with this, A3243G mutation is characterized by strongly reduced copy number of mtDNA in  $\beta$ -cells and other target tissues leading to reduced expression levels of mitochondrial genes. According to recent research, expression level of 25 ribosomal protein genes were strongly reduced in A3243G mutation carrying mitochondria compared with cells having normal copy number of mitochondria.<sup>8</sup>

The A3243G mutation is reported to be present in all tissues, although heteroplasmy levels tend to be high in tissues with a low mitogenic activity, such as skeletal muscle. A3243G mutation is highly associated with altered glucose metabolism and low insulin secretion from  $\beta$ -cells but, shares low frequency of association with insulin resistance. This form of mtDNA is found in varying frequency in different ethnic populations. As it was reported that the prevalence of this mutation was within a range of 1-3% in Japanese diabetic populations<sup>10</sup> and 2% in the French population.<sup>11</sup> Additionally, as per study from North Indian patients, it was found to be present in only 1% (out of 96) of patients.<sup>12</sup> Similarly a study from Coimbatore also revealed that only 1.3% of diabetic patients (out of 150) were carrying A3243G mutation in their mtDNA.<sup>13</sup> All these studies indicate towards the lower prevalence of this form of diabetes. Probably because of its overlapping features with other type of diabetes, it is not properly diagnosed as mtDB and only few reports are available on this form of diabetes, which is misinterpreted as low prevalence of mtDB. However, it can be differentially diagnosed if one has good clinical acumen and high index of suspicion, as discussed in next section.

### DIFFERENTIAL CLINICAL CHARACTERIZATION OF mtDB FROM OTHER FORMS OF DIABETES

Though mtDB share some features with other form of diabetes, but it is clinically distinguishable with respect to several characteristics from the two idiopathic forms of diabetes.

T1D is an autoimmune disorder, characterized by low insulin secretion from pancreatic  $\beta$ -cells or glucose intolerance. It progresses at the age of 15-39 years, more prominently during childhood and young period of life cycle. The reduction in levels of insulin secretion results through mitochondrial dysfunctioning due to overproduction of ROS. T1D is referred as an autoimmune disorder because of two factors: (1.) Cytotoxic activity of NO (nitric oxide) which triggers apoptosis or cell necrosis by inhibiting cytochrome oxidase and ultimately results in  $\beta$ -cell damage.<sup>5</sup> (2.) Over ROS production and  $Ca^{2+}$  over flux in mitochondria that lead to the opening of MPTP (mitochondrial permeability transition pore) resulting in mitochondrial swelling, release of cytochrome C and cell damage.<sup>5</sup> Hence, T1D is linked to low insulin secretion which develops predominantly during childhood and young period of life and is not characterized by development of any clinical disorder.

T2D or adult-onset diabetes is characterized by insulin resistance to insulin target tissues (muscle, adipose tissue, and liver) with relative low insulin secretion from  $\beta$ -cells. Insulin resistance is a physiological condition where insulin becomes less effective in lowering blood glucose levels because of mitochondrial dysfunctioning that inhibits the autophosphorylation of molecules of IRS (insulin receptor substrate family) during the interaction of insulin molecules to insulin receptors located at the membrane of target tissues.<sup>7</sup> Hence, the T2D is characterized by insulin resistance with low insulin secretion, hepatic dysfunctioning, obesity (lipid accumulation) and decreased skeletal muscle glucose uptake without any specific clinical disorder. MODY, the heterogeneous group of monogenetic disorder is characterized by defective mitochondrial function in  $\beta$ -cell. This form of diabetes mostly develops due to mutation in gene encoding Glucokinase and nuclear transcription factors like Hepatocyte nuclear factor1a (HNF1A) and Hepatocyte nuclear factor4a (HNF4A), resulting in impaired insulin secretion and hyperglycemia.

However, mtDB differs from T1D, T2D and MODY on these grounds as summarized in following table:

Thus based on following parameters, mtDB can be differentially diagnosed:

- 1) mtDB has maternal transmission of disorder & surely gets transmitted to zygote where mother is a mt-diabetic patient while this is not a case with T1D, T2D or MODY form of diabetes.
- 2) A3243G mutation results in phenotype similar to T1D and includes impaired insulin secretion, altered glucose metabolism in skeletal muscle and increased gluconeogenesis due to overproduction of lactate; however it can be

Characteristics	T1D	T2D	MODY	mtDB
Age of Onset	15-39	Above 40	Before 25	Above 35
Insulin Secretion/Insulin Resistance	Low Insulin Secretion	Variable Insulin and Insulin Resistance	Low insulin secretion	Low insulin secretion
Inheritance Pattern (maternal/paternal)	Maternal /Paternal	Maternal /Paternal	Maternal /Paternal	Maternal
Autoimmune etiology	Yes	No	No	No
Genetics	Polygenic	Polygenic	Monogenic (HNF4A or GCK)	Monogenic (only mt tRNA <sup>Leu(UR)</sup> )
Association with Obesity	No	Strong	Rarely Obese	No
Additional Clinical Syndromes	Ketoacidosis with hyperglycemia	Microvascular and Macrovascular complications (Cardiomyopathy)	No Additional Syndrome	MELAS, Cardiomyopathy, Deafness and Retinopathy

differentiated based on inheritance pattern, genetic screening and age of onset.

In some patients if the age of onset is similar to T1D, then it can be misdiagnosed but the person will not show the characteristic antibodies associated with autoimmune T1D.

- 3) Development of clinical disorders like cardiac dysfunctioning, MELAS syndrome, deafness etc.
- 4) mtDB is different from MODY because of maternal inheritance and comorbidities like cardiomyopathy, renal failure, deafness and MELAS etc.

### ADDITIONAL CLINICAL FEATURES OF MITOCHONDRIAL DIABETES

Mitochondrial diabetes may come with other organ problems and hence shares the consistent association with symptoms like retinopathy, cardiomyopathy, renal dysfunction, deafness, myopathy, cerebellar ataxia, or other unusual neurological features.

**Mitochondrial encephalomyopathy with lactic acidosis** and stroke-like episodes (MELAS) is a progressive neurodegenerative disorder characterized by stroke-like episodes due to focal brain lesions (in parietooccipital lobes), lactic acidosis and mitochondrial myopathy. It is the most frequently occurring maternally inherited disorder, progresses at the age of 15 years. MELAS syndrome is characterized by different biochemical and morphological abnormalities in mitochondrial genome which are associated with at least 6 different point mutations, 4 of which are located in the same gene, the tRNA<sup>Leu(UR)</sup> gene. The most common mutation, found in 80% of individuals with MELAS, is an A-G transition at nucleotide (nt)-3243 in the tRNA<sup>Leu(UR)</sup> gene.<sup>14</sup> An additional 7.5% have a heteroplasmic T-C point mutation at base pair 3271 in the terminal nucleotide pair of the anticodon stem of the tRNA<sup>Leu(UR)</sup> gene. Down expression of human mitochondrial leucyl-tRNA synthase (LARS2) develops the primary defect (inefficient aminoacylation capacity of tRNA) that leads to reduced expression of complex 1 and complex 3 encoding genes.

Hence, the most common A3243G mutation targets mitochondrial functioning that not only affects the  $\beta$ -cell's insulin release activity but also target multisystemic organ system involving central nervous system, skeletal muscle and eye by development of MELAS syndrome.

**Cardiac Dysfunctioning** : Cardiac dysfunctioning or development of cardiovascular disorders is a major after-effect of mitochondrial diabetes. Reduced action of insulin on cardiac cells diminishes the uptake efficiency of glucose, which reduces the glucose utilization and limits the oxygen consumption for ATP synthesis and finally develops the state of cardiomyopathy. There are two mechanisms that govern the cardiovascular dysfunctioning and development of cardiovascular disorders during mitochondrial diabetes.

- 1) **Lipotoxicity**: Altered rate of  $\beta$ -oxidation in cardiomyocytes stimulates the peroxidation of free fatty acid molecules by ROS and results in release of lipid peroxides. These cytotoxic molecules damage cardiac cells and leads to apoptosis.<sup>5, 15,16</sup>
- 2) UCPs: UCP2 & UCP3 are expressed in heart and are activated by increased production of ROS, lipid peroxides and free fatty acids. Activated UCPs increase mitochondrial uncoupling rate and increase oxygen consumption inside cardiac cells without ATP production that, diminishes cardiac efficiency.<sup>15</sup>

**Deafness**: Phenotypically mtDB is combined with deafness in more than 60% cases and bilateral hearing impairment is a consequence of A3243G mutation of

the tRNA<sup>Leu</sup>(<sup>UUR</sup>). This type of deafness often becomes evident shortly before the diabetes appears. For that reason, this kind of diabetes is also called MIDD (Maternally Inherited Diabetes with Deafness). As per the study, the association between diabetes, deafness and mitochondrial mutations is well recognized and involved the presence of two point mutations as A3243G and T14709C. The association is strong with A3243G mutation that among 1% diabetic patients, 0.3% of them are harbouring deafness with A3243G mitochondrial mutation.<sup>17</sup> The mutation restricts the ATP production in strial marginal cells of inner ear. The strial marginal cells, located near the organ of Corti are rich in mitochondria where the impaired ATP production results in reduction of K<sup>+</sup> ion concentration of endolymph, which are needed for the outer hair cells of the organ of Corti to amplify the sound waves at high frequencies. The diminished rate of energy production and its related consumption produces neurosensory deafness.<sup>17</sup>

**Diabetic Retinopathy:** Diabetic retinopathy is a microvascular disorder that develops due to mitochondrial dysfunctioning (increased ROS production and reduced ATP synthesis) which is a result of heteroplasmic A3243G mutation in mt genome.<sup>18</sup> As, it has been already discussed above, A3243G mutation restricts the mitochondrial activity by lipid accumulation in mitochondria that stimulates ROS production which alters the mitochondrial membrane potential and allows cytochrome C to move out from mitochondria. The decreased amount of cytochrome C restricts the ATP synthesis process and diminishes the respiration rate inside retinal cells. Along with this, ROS activates MMP2 gene in retinal myocytes that act as proapoptotic agent as its activation cleaves nuclear PARP that further induces the release of apoptosis inducing factors from mitochondria. Release of apoptosis inducing factors like MT1-MMP sensitizes endothelial cells to apoptosis by caspase 3 activation. Up-regulation of MMP2 in retina accelerates the loss of capillary cells resulting in development of diabetic retinopathy.<sup>18</sup>

## THERAPEUTIC APPROACHES

As, mitochondria is proven to be a central player in regulation of insulin secretion and its action, hence therapeutic treatment/management by targeting the mitochondria could be another alternative for possible cure of this form of diabetes. Excessive ROS production restricts the mitochondrial activity by posing the great damage to mitochondrial DNA, proteins and lipids. Hence, mitochondrial activity could be improved by controlling the level of oxidative stress by following ways:

**Coenzyme Q:** CoQ, an electron carrier acts as antioxidant molecules in mitochondria in its reduced form either by regeneration of active ascorbic acid and tocopherol or by directly reacting with peroxy-nitrite.<sup>5,7,8,19</sup> CoQ in its semiquinone form acts as chain breaking agent and protect the cell from lipid peroxidation. CoQ shares some signaling properties like inducing UCP activity and enhancing the expression of regulatory proteins for mt biogenesis. But according to studies, CoQ concentration was found to get reduced in diabetic state of patient which not only restricts the ATP production through OXPHOS but also diminished the antioxidant effect of CoQ.<sup>5</sup> Hence the administration of ubiquinone with its antioxidant properties attempts to prevent and control the complications of diabetes.

**lipoic acid:** lipoic acid and its reduced form dihydrolipoic acid are powerful antioxidants.  $\alpha$ -lipoic acid scavenges hydroxyl radicals, hypochlorous acid, and peroxy-nitrite and reduces the extent of oxidative damage to cell. Dihydrolipoic acid also scavenges superoxide and peroxy radicals and can regenerate other antioxidants including vitamin C, vitamin E and glutathione. Along with antioxidant property,  $\alpha$ -lipoic acid increases glucose uptake by target cells through recruitment of glucose transporters (GLUT) to their cell membrane.<sup>5, 7, 19</sup> Administration of  $\alpha$ -lipoic acid not only improves the insulin stimulated glucose uptake, rather it prevents the cell from oxidative damage as well.

**N acetylcysteine (NAC):** It is a potential antioxidant, reacts with multiple radical species and forms NAC disulphide as end product. NAC disulphide penetrates cells where thiol group confers antioxidant property by transferring electrons from SH group to hydrogen peroxide, resulting in effective removal of hydrogen peroxide. NAC also exerts antioxidant property by increasing the formation of GSH (glutathione) and GPx (glutathione peroxidase) that protect cells from oxidative damage.<sup>5</sup>

**Exercise and highly nutritious diet:** Exercise and nutrient full diet decrease the risk of mitochondrial dysfunctioning and diabetes. Physical activity improves glucose tolerance by increasing energy production in cells and insulin secretion from beta cell. Exercise offers several benefits like stimulation of factors, that increase the rate of mitochondrial biogenesis and improves insulin sensitivity to target cells.<sup>5,8,19</sup> Full nutrient enriched diet should be an inclusive of all required

vitamins, minerals and compounds that play an important role in maintaining mitochondrial structure and enhancing mitochondrial activity and its functions.<sup>19</sup>

Hence, the antioxidant chemical compounds, vitamins, minerals and physical activity focus on mechanisms that regulate mitochondrial biogenesis, ROS production and respiration.

## CONCLUSIONS

Mitochondria, the centre of energy production and its metabolism represent itself as the central crossroad of various metabolic pathways. One of the discussed metabolic pathways is insulin exocytosis and its secretory regulation where mitochondria generate the numerous signals that regulate the mediators of cellular excitability and insulin exocytosis from beta cell. Glucose stimulated insulin release from beta cell's mitochondria solely depends on ATP/ADP ratio and ROS production levels. Increased ATP production and diminished ROS levels stimulates the insulin exocytosis from beta cells. But the disturbance in this ratio of ATP production to ROS levels along with defected mitochondrial morphology and biogenesis contributes to increased oxidative load to mitochondria or mitochondrial dysfunctioning which not only interferes with insulin secretion but also results in development of metabolic disorder like diabetes. As the epidemiology of diabetes progresses, a new form of diabetes comes in picture where it is not only remain restricted to mitochondrial dysfunctioning as leading cause rather come out as a causative effect of mtDNA mutation (A3243G tRNA mutation). This form is referred as mitochondrial diabetes characterized with several distinguishing features like age of onset, maternal inheritance and several clinical disorders like deafness, MELAS, cardiac dysfunctioning etc. As discussed, the variable phenotype and its several overlapping clinical features with other types of diabetes, makes its diagnosis and genetic counseling very difficult. However suspicion for mitochondrial diabetes should be in more when the cases with strong familial clustering and maternal inheritance are observed.

For the management and possible therapy of mtDB, all the factors like ubiquinone Q,  $\alpha$ -lipoic acid, N acetylcysteine etc which can enhance the mitochondrial activity could be an alternative. However, certain care also needs to be taken while prescribing common anti diabetic drugs to these patients. Metformin (a common oral drug given to T2D patients) should not be recommended to these mtDB patients. Reason behind this is, in mtDB mitochondrial failure can cause rise in lactic acid and metformin is also known to induce lactic acidosis. Hence, mtDB patients taking metformin are much higher at risk of lactic acidosis.

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