Maternally Inherited Type 2 Diabetes and Deafness: Clinical and Molecular Aspect in Pakistan

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Abstract

Background: A3243G mutation in tRNA Leu (UUR) leads to a specific clinical syndrome characterized by diabetes and sensorineural hearing defect, hence called as “Maternally Inherited Diabetes and Deafness (MIDD)”. Methods: The study was retrospective, analytical case control study. Non-probability convenient sampling technique was used. Subjects were divided into two groups. Thirty-nine patients with phenotype of mitochondrial diabetes and a strong maternal history of diabetes (group 1) and 40 non-diabetic individuals (controls) with no maternal history of diabetes (group 2). MtDNA, isolated from peripheral blood leukocytes, was analyzed to detect A3243G tRNAleu(UUR) gene mutation.

Results: In spite of the presence of characteristic features of maternally inherited diabetes and deafness, A3243G tRNAleu(UUR) gene mutation was absent in patients and controls. Mean age of onset of diabetes was 34.06 years and of deafness was 47.56 years. Mean plasma glucose, HbA1c, cholesterol and triglyceride level in patients was significantly higher (p< 0.001) whereas body mass index (BMI) of diabetics was significantly low (p< 0.001) as compared with controls.

Conclusion: Although A3243G tRNAleu(UUR) gene mutation was not likely to be a frequent cause of mitochondrial diabetes in selected group of patients, other mitochondrial diabetogenic mutations as well as mechanisms involved in pathogenesis of mitochondrial diabetes must be considered.

Key Words: Mitochondrial DNA (mtDNA), maternally inherited diabetes and deafness (MIDD), body mass index (BMI), transfer RNA (tRNA).

Introduction

Diabetes Mellitus (DM) is a frequently occurring, multifactorial disorder with increasing pandemic frequency. Surprisingly, for the last few years, a new trend in development of type 2 diabetes mellitus (T2DM) has been noticed i.e. onset of diabetes at the age of 30 - 40 years, instead of late 40s and secondly, diabetics with early onset of T2DM have low body mass index. The genetic cause of early onset of diabetes has been a hot point of research for several decades and is yet to be explored further but the progression in this domain has been facing many difficulties making the situation complicated. In addition to nuclear genome, mitochondrial genome has been studied thoroughly for the last few decades to find out its association with the pathogenesis of type 2 diabetes mellitus.

Ubiquitous intracellular organelles, mitochondria, are involved in many metabolic pathways including energy generation by oxidative phosphorylation (OXPHOS) to keep the cells alive and functional. OXPHOS generates energy rich phosphate compounds i.e. Adenosine Triphosphate (ATP). ATP provides energy to all metabolic processes and physical activities going on. In addition, ATP also plays a pivotal role in the synthesis and release of insulin by pancreatic beta cells, as β cells are sensitive to hyperglycemia. Raised blood glucose alters the intracellular ATP/ADP ratio and triggers the release of insulin. Hence, ATP generation in pancreatic β cells is essential for its normal functioning. Most of the components of mitochondrial respiratory chain complexes are encoded by nuclear genome. However, few proteins subunits of mitochondrial respiratory chain complexes, are encoded by mtDNA. In 1963, Nass and Nass demonstrated presence of extrachromosomal DNA within mitochondria called “Mitochondrial DNA”, (mtDNA).

Mutations of mtDNA are involved in several disorders and diabetes mellitus is a common and the predominant hallmark associated with various mitochondrial diseases. Approximately twenty mtDNA mutations have been detected to be associated with mitochondrial diabetes, A3243G tRNAleu(UUR) gene mutation being the most common diabetogenic heteroplasmic point mutation in mtDNA. This mutation may lead to loss of mt transcription, translation and ultimately disturbed function of respiratory chain. It has been detected in 0.5–1.5 % of the diabetics with T2DM.
accompanied with sensorineural hearing defect - a new subtype of diabetes called “Maternally Inherited Diabetes and Deafness (MIDD)”. Accompanying deafness may be due to the fact that the cells in the cochlear portion of the ear are also susceptible to energy deficit. Hence, any disruption in ATP production in cochlear cells can affect the normal hearing power of the patients with mitochondrial diabetes. This leads to the development of “Sensory-neural deafness” along with diabetes. Hence, due to the combination of these two features, this disease got its present name of MIDD.

Other features of maternally inherited mitochondrial diabetes are early onset of diabetes i.e. age ≤ 40 yrs, insulin dependence at later stage, low or normal body mass index (BMI) and sensorineural hearing defect. The clinical features of mitochondrial diabetes linked with A3243G mutation has been discussed thoroughly by Chen YL et al. (2004).

Regarding the mtDNA gene mutations associated with mitochondrial diabetes in Pakistan, no data is available. Therefore, this study was conducted to ascertain the presence of the most common diabetogenic mutation i.e. A3243G mutation in tRNALeu(UUR) gene in the selected group of patients who presented with typical characteristic features of maternally inherited diabetes and deafness.

**Patients and Methods**

This study was analytical case control study. Patients were selected from Military hospital, Rawalpindi, Combined Military hospital, Multan and rural areas near Chakri Road, Rawalpindi. This study was conducted from January 2005 to December 2008. Inclusion criteria were bilateral sensorineural deafness, onset of diabetes before the age of 40 years and insulin dependence, either at the time of diagnosis or later on. Audiometric services of the mentioned hospitals confirmed sensorineural hearing loss. Sample size was 79. Subjects were subdivided into two groups, non-diabetic controls with no maternal history of diabetes (n= 40) and patients with MIDD phenotype (n= 39).

Molecular study was done at Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi. After getting written consent from the subjects, samples were scanned for the detection of potential mutations within the region of mitochondrial DNA tRNALeu(UUR) gene (np 3035 - 3456, 422 bp fragment) which contains the whole sequence of tRNALeu(UUR) gene.

The genomic DNA was extracted from peripheral - blood leukocytes by Kit method (GENTRA, USA) followed by whole genomic amplification of mtDNA by using standard kit method (Qiagen Repli – G mitochondrial DNA kit). The required mtDNA fragment was amplified by PCR with AmpliTaq DNA polymerase. Two sets of primers were used. Forward Primer was 5’- CGTTTGTITCAACGATTAAAG -3’ and Reverse Primer was 5’- AGCGAAGGGTTGTACTAGCC –3’.

The amplified mtDNA 422 - bp fragments were digested by restriction enzyme Apal (Fermentas Life Sciences) to identify any A3243G mutation followed by electrophoresis. The amplified PCR product was to split into 210 bp and 212 bp fragments if it had A3243G mutation to constitute the recognition site for Apal. The PCR products were sequenced directly (Beckman Coulter, USA).

Plasma glucose was estimated by enzymatic colorimetric method using glucose oxidase enzyme to oxidize glucose. Glycosylated hemoglobin (HbA1c) was determined by micro column method (ion exchange chromatography). Plasma cholesterol and triglyceride level was determined by Enzymatic colorimetric method.

Audiography determined type and extent of hearing loss.

Body mass index (BMI) was calculated by dividing Weight (Kg) by Height (m2).

Statistical data was analyzed by computer software programme “Statistical Package for Social Sciences (SPSS)” for windows, version 15.00 using Independent - Sample T-test. The results are expressed as mean ± s.e.m. p-value less than 0.05 was taken as statistically significant.

**Results**

A3243G tRNALeu(UUR) gene mutation was not detected in two study groups (figure 1). Direct sequencing also confirmed these negative results (fig 2 & 3).

Mean age at which the patients developed diabetes mellitus was ± 34.06 years and developed deafness at ± 47.56 years. BMI was significantly low (p< 0.001), mean plasma glucose level and glycosylated hemoglobin level was significantly higher (p < 0.001) in diabetics as compared with controls. Plasma cholesterol and triglyceride level of patients was significantly higher (p< 0.001) as compared with controls (Table 1).
In this paper, the results of the search of A3243G mutation in maternally inherited type 2 Diabetes in Pakistani population are reported. Though mtDNA A3243G mutation in the tRNA^{Leu(UUR)} gene could not be detected in the selected group of study but it is really difficult to reach a conclusion at this moment because an interaction of wide range of variants in mitochondrial DNA, nuclear DNA as well as environmental factors is involved in the development of MIDD.

### Table 1
Comparison of various parameters of Group 1 with Control Group.

<table>
<thead>
<tr>
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<th>Patients with MIDD (n = 39)</th>
<th>Controls (n = 40)</th>
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<tbody>
<tr>
<td>Age at onset of diabetes (years)</td>
<td>34.06 ± 5.23</td>
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</tr>
<tr>
<td>Age at start of deafness (years)</td>
<td>47.56 ± 6.4 (mean ± SD*)</td>
<td>-----</td>
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<tr>
<td>Body Mass Index (BMI)</td>
<td>20.06 ± 0.29**</td>
<td>24.52 ± 0.53</td>
</tr>
<tr>
<td>Plasma Cholesterol (mmol/L)</td>
<td>6.054 ± 0.24**</td>
<td>4.78 ± 0.15</td>
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<tr>
<td>Plasma Triglyceride (mmol/L)</td>
<td>1.78 ± 0.04**</td>
<td>1.32 ± 0.04</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>13.36 ± 0.94**</td>
<td>5.13 ± 0.08</td>
</tr>
<tr>
<td>Glycosylated hemoglobin (mmol/L)</td>
<td>9.45 ± 0.42**</td>
<td>5.55 ± 0.10</td>
</tr>
<tr>
<td>Glycosuria(%)</td>
<td>80 %</td>
<td>Absent</td>
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* SD: standard deviation
**p < 0.001 as compared with normal control subjects

Moreover, coordinated action of two genetic sources leads to the biogenesis of functional mitochondria i.e. nuclear genome and mitochondrial genome. Therefore, any defect in one of these genetic sources may contribute in the pathogenesis of mitochondrial diabetes. In addition, the level of heteroplasmy varies among different tissues being the highest in postmitotic tissues like pancreas, brain and skeletal muscles, and the lowest in rapidly dividing cells, such as blood leukocytes. Mitochondrial DNA was isolated from peripheral blood leucocytes. Hence, we cannot overlook the probability to miss the diabetogenic mtDNA mutation. Moreover, in 2001, similar study carried on Polish Population revealed that out of 129 patients with maternally inherited type 2 diabetes, none of the patients was identified as a carrier of A3243G mutation. In addition, two studies on South Indian adult patients with maternally inherited type 2 diabetes also did not report A3243G mutation. Nevertheless, the rising prevalence of MIDD in Asian countries cannot be underestimated, as recently Sahu et al (2007) found mitochondrial DNA A3243G mutation in ~1% Asian subjects.

Patients in our study group presented with
deafness for loud sounds, a characteristic feature of sensorineural hearing defect. No doubt, that the expected genetic cause responsible for MIDD phenotype was not found, but to our knowledge, no other possible mechanism explains the etiology of deafness associated with maternally inherited type 2 diabetes.

Like type 2 diabetes, impaired hearing is also a multifactorial disorder. Several environmental and genetic factors can contribute in its pathogenesis like aging and autoimmune diseases. At the same time, several mitochondrial DNA mutations are associated with hearing loss e.g. A1555G, A7445G, 7472insC and 7751C mutations. It is difficult to pin point the exact cause of deafness at this moment and the role of other etiological factors along with mtDNA mutations (other than A3243G) must be considered.

Another significant finding in our study was low body mass index (BMI) in patients with maternally inherited type 2 diabetes. Although we realize that, the power to detect differences in this study was too small, any proof to show a definite association between MIDD phenotype and low BMI was difficult to provide. However, some recent studies have also shown the involvement of some other mtDNA mutations in the pathogenesis of diabetes, deafness and low BMI e.g. T1095C mutation. Therefore, there might be another significant etiology involved in the association of type 2 diabetes with low body mass index and deafness.

Diabetics with MIDD phenotype belonged to a younger age group. For the last few years, it is seen that mostly patients develop type 2 diabetes in younger age i.e. between 30 – 40 years of age and have low BMI. As few studies conducted in Asia, one report from UK and one from US have also confirmed that a new trend of early onset of diabetes and low BMI is emerging as a significant feature of diabetes. Our study nevertheless provides a support for the hypothesis that mtDNA mutation may be an important genetic factor contributing to low BMI in maternally inherited diabetes.

Thirty-four out of 39 patients (87.18 %) showed the insulin dependence, though initially oral hypoglycemics controlled the blood glucose level. Possible explanation of insulin dependence could be progressively worsening hyperglycemia or gradual increase in insulin requirement. Pronounced age-dependent deterioration of beta pancreatic cells can also lead to decreased insulin production and enhanced insulin requirement. At the same time, other factors like severity of insulinopenia, hyperglucagonemia, lack of dietary education, low socioeconomic status and failure to take recommended balanced diet due to poverty must be considered as well.

Marked dyslipidemia was another characteristic feature seen in diabetics with maternally inherited type 2 diabetes. Several studies conducted in Pakistan have shown that dyslipidemia is a prominent finding in diabetics. Abbasi et al (2007) reported raised plasma levels of cholesterol (total) and low density lipoproteins (LDL) as the only major finding in the Pakistani diabetics, particularly type 2 diabetes mellitus (T2DM). An electrifying rise, in the incidence of diabetes with dyslipidemia, is expected in the near future. Dyslipidemia seen in our study also confirmed the findings of the previous studies in our region.

Our study had certain limitations. Unfortunately, the heteroplasmy of the mutation is the lowest in the peripheral blood leukocytes, most readily available sample, and the highest in the affected tissues. Moreover, there is ~0.7% decline in the heteroplasmy levels in leukocytes per year. Therefore, the chance to detect this mutation was lower in leukocytes and it might have hampered the detection of this mutation. As entire mitochondrial genome is susceptible to pathogenic mutations, comprehensive detection of mtDNA mutation requires sequencing of the entire mtDNA molecule, which could not be done due to lack of funds.

Mitochondrial DNA encoded protein modelling may also be done to detect any alteration in the primary structure of proteins and their possible involvement in maternally inherited type 2 diabetes. Further study of families with non-syndromic hearing loss can lead to the better understanding of association of hearing loss and maternally inherited diabetes mellitus.

The sequencing of the entire mtDNA can rule out the presence of known and novel mutations as well.

**Conclusion**

The A3243G mutation in mitochondrial tRNALeu(UUR) gene cannot be labelled as a main contributing etiological factor of type 2 diabetes in the selected group of patients. The observed clinical hallmarks of diabetes might be the outcome of a combination of several other mechanisms or factors. The exact prevalence of this mutation in Pakistani population can be determined by screening of larger study group.

**References**


