The Genetic Mutation in patients with Type 2 Diabetes Mellitus in Adult

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Abstract: Incidence and prevalence of Type 2 Diabetes mellitus (T2DM) continues to rise among the Egyptian populations. Despite known roles for obesity, sedentary lifestyles and diet, genetic predisposition accounts for a significant risk. It is possible that cell free mitochondrial DNA (MtDNA) exists in the serum of these patients, and these samples might be a source of material for detection of such mutations. A to G transition mutation in mitochondria transfer RNA Leu gene at position 3243 is commonly associated with diabetes mellitus (DM). Diabetes cosegregated with this mutation in a fashion consistent with maternal transmission. The aim of this study is to detect the presence of mtDNA mutation in the serum of patients with type 2 diabetes mellitus, and those with family history of diabetes mellitus. A case control study was conducted among four groups of participants. The first group included 10 patients with diabetes having positive family history of DM in first degree relatives. While, the second group comprised of 10 patients that had DM with negative family history. The third group included non-diabetic subjects with history of DM. The last group comprised of non-diabetics with no family history for diabetes served as control group. Each participant was subjected to comprehensive evaluation and serum samples were taken, and tested for the mt3243 mutation after polymerase chain reaction (PCR) analysis was done. The serum samples then were subjected to APaI digestion to detect any mutation at a certain region along the mtDNA.

Results: PCR amplification of the mtDNA leu (UUR) region in mitochondrial DNA in the serum revealed its presence in all samples. After APaI digestion fragments of amplified DNA fragments, mt3243 was detected in the serum samples of seven patients out of ten With diabetes who had positive family history of diabetes (DM). While, interestingly those with type 2 DM without family history showed positive mutation in six out of the ten patients. In the third group three out of the ten participants, showed to have a positive mutation. While none of the serum samples from healthy subjects revealed such mutations; these finding were of statistical significance (p<0.05).

Conclusions: Therefore, mtDNA and associated mutations are present and detectable in the serum of patients with diabetes mellitus. Hence the serum may be an alternative source for the molecular diagnosis of this mutation associated in diabetes mellitus.

Keywrods: Genetic; Mutation in; patient; Type 2 Diabetes; Mellitus; Adult

1. Introduction
Diabetes mellitus is a complex polygenic disorder; characterized by a disturbance in insulin production or the ability of target tissues to respond to insulin. Several mutations have long been suggested to be expressed in the mtDNA in diabetic patients (Agung, 2005). The use of plasma and serum as sources of genomic DNA for molecular diagnosis has raised interest because if it's non-invasive nature and ease of sample collection. Mutations in the mtDNA have been reported to be associated with a variety of diseases.

It has been proposed that accumulation of mitochondrial DNA mutations in somatic cells contributes to aging and degenerative diseases because DNA genotype influences oxidative damage of its own (Linnane et al., 1989). Studies have demonstrated that tumor specific DNA and fetal DNA are detectable in the plasma and serum of patients with cancer and in pregnant women respectively (Mulcahy et al., 1996).

Pathogenic mtDNA usually exists in heteroplasmic form, with existence of both mutant and wild type mtDNA in affected cells. The degree of heteroplasmy varies considerably in various tissues and among different individuals. For any mitochondrial DNA mutation, the ratio of mutant to wild-type mtDNA (% heteroplasmy) varies across tissues, with low levels in leukocytes and high levels in postmitotic tissues (e.g., skeletal muscle). Direct sequencing is the gold-standard method used to detect novel mutations, but can only reliably detect % heteroplasmy >25%, which is rare in leukocytes (Mathews PM et al, 1994).

An A to G substitution (guanine for adenine) at base pair 3243 in mitochondrial tRNA gene (mt3243) is commonly associated with maternally inherited diabetes and deafness, and other diseases. It is possible that cell free mitochondrial DNA exists in serum and plasma from these patients, and those samples might be a source of material for the detection of such
mutations (Sheng et al., 2000) (Vijaya Padma et al., 2010). This study aimed at studying the presence of mtDNA mutation in the serum and plasma of patients with type 2 diabetes mellitus with positive family history, and those who are non-diabetic but carry a positive family history for DM.

2. Patients and Methods

All subjects were Egyptian patients living in Cairo. Forty patients were included in the present study. The first group was 10 patients with type 2 diabetes mellitus with positive family history; the second group involved 10 patients with diabetes and negative family history for DM, third group had positive family history for DM only and the last group involved 10 healthy subjects who served as a control. The age range for the participants in our study was 28 to 55 years of age. Type 2 diabetes was diagnosed according to the 1985 WHO criteria. A 5 ml blood sample was collected in plain tubes. DNA was isolated from the serum and genotyped for mt3243.

Sample preparation:

Blood samples were collected on plain tubes and were centrifuged at 3000xg for collection of the serum. The serum was centrifuged at 3000xg and collected in new tubes was done to avoid the carryover of blood cells. Samples were then stored at -20°C.

DNA extraction:

100 ul of serum was mixed with an equal volume of digestion solution (20 mM Tris/HCL (pH 8.0), 2 mM EDTA, 0.1% sodium dodecyl sulphate and 0.8 mg/ml proteinase K) and incubated for four hours at 56°C. DNA was then extracted using phenol followed by chloroform. DNA was precipitated with ethanol and sodium acetate and air dried. The precipitated serum DNA were dissolved in 25ul of distilled water, and stored at -20°C.

Mt 3243 genotype was determined by PCR using the primer set 5’AGG ACA AGA GAA ATA AGG CCT3’ and reverse primer used S’AC GTT GGG GCC TTT GCC T A3’. Reactions were carried out in 2 ul volumes containing 2 ul of serum DNA. PCR was preformed for 35 cycles each with denaturation at 95°C for 1 minute, 55°C for 50 seconds, and extension at 72°C for 1 minute. Next, 0.5 uCi of alpha(35 P) dATP was added and one more cycle was performed.

5ul of the PCR products were then digested with 5 U Apal (Gibco BRL, Rockville, Maryland, USA) for two hours at 30°C and then electrophoresed using 8% denaturing polyacrylamide gels at 1000 V for two hours and visualized electronic autoradiography. The presence of mt3243 allowed the 294bp product to be cleaved into 180 and 114 bp fragments. Standards containing 0-100% mutant DNA mt3243 were also included in the assay. The intensity of the bands was measured. The proportion of mt3243 in a sample was calculated by dividing the intensity of mutant bands (114 and 180bp) by the total intensity of both wild type and mutant bands.

Statistical method:

Analysis of data was done by IBM computer using SPSS (statistical program for social science version 12). Chi-square test was used to compare qualitative variables between groups (M. Clinton Miller and- Rebecca G. Kapp, 1992).

3. Results

The characteristic of the subjects in this study are shown in table 1. The age ranged from 28 to 57 for group I and II. While group III the age ranged from 25 to 55, and in group IV the age ranged from 25 to 50. They consisted of 40 participants; among which 10 subjects had positive family history (FH) of DM and had non insulin dependent DM (NIDDM) classified into Group I, and group II were 10 patients who were DM but had a negative FH for DM. While, the third group consisted of 10 patients with positive family history for DM but were not diabetic by history and laboratory investigation. Group IV had neither DM, nor positive FH for diabetes and served as controls (10 healthy participants).

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yrs) Mean/SD</th>
<th>Duration of DM (yrs) Mean/SD</th>
<th>FBS (mg/dl) Mean/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>45±15</td>
<td>10+//-7</td>
<td>165± 8</td>
</tr>
<tr>
<td>Group II</td>
<td>45+1-15</td>
<td>10+//-7</td>
<td>142+//-7</td>
</tr>
<tr>
<td>Group III</td>
<td>49+/+11</td>
<td>10+//-7</td>
<td>100+1-2</td>
</tr>
<tr>
<td>Group IV</td>
<td>40+/+12</td>
<td>10+//-7</td>
<td>90+/-4</td>
</tr>
</tbody>
</table>

Table 1: Clinical characteristics of subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Mutation positive</th>
<th>Mutation negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIDDM with positive FH</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>NIDDM with negative FH</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Non DM with positive FH</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Non DM, negative FH</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Chi-square</td>
<td>X2= 6.2, P &lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

These finding were statistically significant using chi square test X2=6.2, P< 0.05.
4. Discussion

In this study, mtDNA mutation was detectable in the serum samples of seven out of ten patients with type 2 diabetes mellitus and positive family history of maternal DM. While six out of ten had this mutation with negative, family history for DM but had DM. In addition, those with positive family history of DM but did not have DM, three out of ten showed to have mutation. Kadowaki et al in 1994 suggested that this mitochondrial mutation should be considered in cases of slowly progressive IDDM or insulin deficient NIDDM.

Previous studies have shown that tumour specific DNA and fetal DNA are present in plasma and serum from patients with cancer and pregnant women (Nawroz H. et al., 1996). However, Reardon et al. in 1992 described two patients with mutation of mtDNA who had poor insulin secretory responses to glucose. Similarly, Kadowaki and colleagues in 1994 demonstrated a significant reduction of maximal insulin secretory capacity and early secretion response of insulin to glucose administration in diabetic patients with tRNA Leu(UUR) 3243 mutation. Moreover, it was found that this mutation may cause gradual B-cell destruction, but no direct correlation was found between the percentage of heteroplasmy with the age of onset of diabetes, suggesting the pathogenic importance of peripheral insulin resistance. It is possible that this phenotype variability among the studied groups could be related to the different expression of the mutant genotype at different times with different thresholds (Lee et al. 1996). Therefore, mitochondrial genes are possible causes for both IDDM and NIDDM (AT Choo-Kang 2002).

Alcolada et al in 1994 and others have shown that in the diabetes with mitochondrial DNA mutations, the sensing pathway may be intact, and the prevailing hyperglycemia results in increased concentrations of insulin precursors, typically seen in those with NIDDM and impaired glucose tolerance. Therefore, close observations might provide an opportunity to study the onset and natural course of Diabetes with tRNA Leu (UUR) 3243 mutation (Sue CM et al. 1993) and (Kanamori A et al. 1994). Several families have been described in which a mutation of mitochondrial DNA, the substitution of guanine for adenine (A-to-G) at position 3243 of leucine transfer RNA, is associated with diabetes mellitus and deafness. The prevalence, clinical features, and pathophysiology of diabetes with this mutation are largely undefined (Takashi Kadowaki, et al., 2008).

According to a study done by Kumiko O. and colleagues (2001) found that A3243G mutation was responsible for a deterioration in insulin secretion, by pancreatic B cells which demonstrated highest percentage of heteroplasmy.

Heteroplasy which is the ability of cells that can harbor a mixture of mutant and wild type mitochondrial DNAs. Also it was found in the previous studies that the degree of heteroplasy determines the age onset of diabetes in patients with A3243G mutation. Therefore, the diagnosis of A3243G mutation before the hyperglycemic state develops seems to be very important in female patients not only to prevent, hyperglycemia, but also to prevent abnormal pregnancy (Yanagisawa K. et al., 1995). In addition, to a study done by Rinki S et al., in 2006 who stated there is some correlation between the degree of heteroplasy with the age of onset of diabetes and the severity of deafness. Heteroplasy levels in the muscle may better correlate with the degree of age corrected hearing loss (Ulmonen S et al., 2001).

In this study patients who had this mutation had sudden onset of hyperglycemia and showed rapid progression to insulin dependent state. A to G transition mutation in mitochondria transfer RNA Leu gene at position 3243 is associated with stroke like episodes (Remes AM et al., 1993), myoclonic epilepsy with red ragged fiber. However, Suzuki and colleagues in 1994 found A to G transition mutation in mitochondrial transfer RNA Lys gene at position 8344 and also other mitochondrial cytopathies are associated with diabetes mellitus. Other studies found that tissues from several diabetes mellitus patients showed extremely high levels of A-G transition mutation, and concluded that mitochondrial DNA A3243G mutation is one of the major causative factors of mitochondrial diabetes mellitus (Shinji 2008).

Latent autoimmune diabetes in adults (LADA) is a form of type 1 diabetes which is often diagnosed and treated as type 2 diabetes (T2DM). People with LADA show slow progression to insulin dependence and can be distinguished from T2D by blood tests for glutamic acid decarboxylase
autoantibodies (GADA). Routine glutamic acid decarboxylase autoantibody (GADA) testing should be performed in all those presenting at ages 18-45 years and genetic investigations can be targeted to phenotypically defined subjects. The finding of a specific etiology will allow individualization of management and give patients valuable information about their condition (Katharine R. Owen et al 2003).

Many mitochondrial mutations have been suggested to be associated with diabetes mellitus, however only A3243G substitution in the tRNA leu gene is in fact firmly established to be causal for DM (Campos et al., 1995). Others are only provisionally associated with DM because they have been found in single cases only, albeit syndromic with other neurological signs common to mitochondrial disorders, or are a subject to controversy they are found also in normal population (Agung P 2005) (Mezghani et al 2013). Thus, diabetes mellitus patients with Mitochondrial DNA A3243G Mutation may show a wide variety of clinical features.

We conclude, therefore, that screening for mtDNA mutations should be considered in patients with maternally inherited diabetes, but may be more helpful when additional features of mitochondrial disease are present. The observations that have been found in the present study suggest that alterations of mitochondrial DNA may to some degree contribute to the development of diabetes mellitus. Mutation detection may also be important for patient management and identification of liable family members for future development of DM.

References


