Evaluation of clinical, biochemical, chromosomal and micronuclei frequency in type II Diabetes Mellitus patients: A case control study

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Abstract
Diabetes Mellitus (DM) is a chronic disease due to elevated level of blood glucose which can lead to complications in many parts of the body. International Diabetes Federation has estimated that DM patient numbers will increase to 123.5 million by 2040. Diabetes and its complications include blindness, nerve damage, heart attack and kidney failure and they depended on the biochemical and immunological parameters. The candidate genes were playing a vital role in the development of DM. The prime aim of the study was to examine the clinical, biochemical, chromosomal and micronuclei frequency in Diabetes Mellitus patients. In the present study, 120 experimental and 120 controls subjects were recruited, they were further divided in to two groups in accordance with the age and disease duration. Peripheral blood samples were used to analyze the clinical and cytogenetic parameters.

Result of the study was analyzed based on various clinical parameters. It is found that the total cholesterol level and micronuclei frequency were significantly higher compared to the controls and insignificant levels of creatinine and chromosome damages were found in higher disease duration type of diabetic patients. The assessments of clinical, biochemical and chromosomal alterations indicate the risk of organ failure and the higher value was influenced by the duration of disease.

Keywords: Diabetes, Clinical, Cytogenetic, Glucose, Cholesterol.

Introduction
Diabetes is a chronic disease due to elevated level of blood glucose which can lead to complications in many parts of the body and it has the most frequent complications with high morbidity and mortality4. Diabetes Mellitus is categorized into two types, type 1 and type 2. Type 1 is insulin dependent diabetes mellitus (IDDM). Another one is non-insulin dependent diabetes mellitus (NIDDM), which is rather silent, chronic, often unidentified killer, mostly among the adult population and it can be prevented.

Diabetes is not a terminal condition but it is combined with risk factors like heart attack, kidney failure, blindness and nerve damage which can increase premature morbidity and mortality that means a reduced quality of life and reduced life expectancy. In recent years, International Diabetic Federation has estimated an increase in Diabetic patients from 19 million in 1995 to 66.8 million in 2015. These reports suggest an increase in number of patients to 642 million by 204010.

WHO 2019 reports are indicating that 74% of deaths accounted for non-communicable diseases and diabetes resulted in 1.6 million deaths, thus becoming the 9th leading cause of death globally44. Tao et al42 predicted that nearly 592 million people are to die due to the diabetes by the year 2035. IDF24 reported that diabetes has reached epidemic proportions in China and India.

Hematological and biochemical factors are significantly influenced by the diabetic patients and the risk factor for atherosclerosis and coronary heart disease22. Type 2 DM and obesity persons have the higher LDL cholesterol and lower HDL cholesterol8. Oxidative stresses are leading to DNA damage which is potential link to diabetic complications22. ROS-mediated modifications include damage to the cells, tissues or organs and promote the mechanism of several diseases7. An increased production of oxygen-derived free radicals and the decrease in the activity of free radical scavenger system have been reported in diabetes20. ROS could cause DNA damage16 and biomarker for cancer risk11. Salah et al37 reported that DM has the ability to induce DNA damage.

Numerical aberrations involve the loss or gain of entire chromosome, giving rise to monosomy or trisomy. Structural aberrations affect parts of chromosomes, usually implying a break in the chromosome and give rise to different rearrangements of chromosomes leading to major causes of genetic disorders3. The most suitable genotoxicity tests include chromosomal aberrations (CA) and micronuclei (MN) test11,44. Chromosome aberrations such as dicentries, polycentrics, rings and acentric fragments are shown in damaged cells30.

Material and Methods
The study has recruited 120 experimental and 120 controls subjects. They were further divided in to two groups in accordance with the disease duration. The study was ethically approved by Dhanvantri College of Nursing, Medical Review Committee Board, Tiruchengode, Namakkal, Taminadu. Written consent form was obtained from all the subjects. Information regarding health, habit,
profession and disease duration was recorded using the standard questionnaire. Peripheral blood samples were collected from the vacutainer tubes and used to analyze the clinical and cytogenetic parameters.

**Biochemical parameters:** The FBS, HbA1c, total cholesterol, LDL-C, HDL-C, VLDL-C and creatinine levels were estimated by the conventional colorimetric methods in SRL laboratory.

**Cytogenetic study**

**Chromosome aberration assay:** Chromosome cultures were initiated by the protocol of Hoyos et al.\(^{23}\) methods. A volume of 0.5 ml of blood was added to 4.5 ml of HiKaryoXL™ RPMI Medium w/L-Glutamine, FBS, PHA-M, Penicillin, Streptomycin and NaHCO₃. The chromosome culture was incubated at 37°C for 72hrs and the culture tube was shaken two times per day. After 71 hrs, chromosome cultures were treated with 0.01mg/ml colcemid solution to arrest cells at the mitotic stage. Chromosomes were harvested after 72hrs. The cells were treated with Carnoy’s fixative (3:1 ratio of methanol: acetic acid) twice. Slides were prepared and stained with Giemsa stain. For the CA analysis, 50 metaphase chromosome plates were analyzed for all subjects under the Leica light microscope (100X) and chromosome spread was photographed.

**Micronuclei in peripheral lymphocytes:** Cytokinesis blocked blood micronucleus assay was carried out by the method of Fenech and Morley.\(^{17}\) 0.5 ml of blood was added to 4.5 mL of HiKaryo XL™ RPMI Medium w/L-Glutamine, FBS, PHA-M, Penicillin, Streptomycin and Sodium bicarbonate. The chemical combination was incubated at 37°C for 72 hours. Cytokinesis was blocked by the addition of cytochalasin B at a final concentration of 6µg/ml 44hrs after stimulus with PHA. Cells were collected after 72 hrs of incubation and treated for 1 min in hypotonic solution (0.075 M KCl), then fixed with methanol and acetic acid (3:1) fixative solution, this step was repeated two times after 20 mints, slides were stained with Giemsa staining.

**Statistical analysis:** All the data were subjected for Analysis of Variance (ANOVA) followed by DMR test and Bonferroni’s correction at the probability level of p<0.05 using SPSS-16 version.

**Results**

The general characteristics of the participants are listed in the table 1. The study was classified in two groups in accordance with the age and disease duration. The data represented that the mean age of group I experimental subject was 39.77±5.32 years and the age ranged from 28-40 years. Group II experimental subjects mean age duration was 52.37±7.08. Range was above 40 years. The experiments subject included II DM patients after the confirmation of glucose (FBS) and HbA1c test and higher BMI (basal metabolic rate) leveled samples.

Biochemical investigation indicated the varied level of lipid profiles and creatinine level in both subjects (Table 2 and graphs 1-6). Total cholesterol level was significantly elevated in group I DM experimental subjects (221.74±39.31) when compared to the normal controls subjects. The HDL-C level was insignificantly (44.31±6.60) decreased when compared to controls and normal in reference range. LDL-C level was significantly (Group II: 111.24±18.59) elevated when compared to the controls and lower aged groups. VLDL-C level and creatinine levels were insignificantly different in experiments from the control groups.
Table 1
Demographic details of diabetes mellitus patients.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Experimental subjects Mean ± SD</th>
<th>Control subjects Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total samples</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Male</td>
<td>68 (56.66)</td>
<td>68 (56.66)</td>
</tr>
<tr>
<td>Female</td>
<td>52 (43.33)</td>
<td>52 (43.33)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I (age below 40)</td>
<td>39.77±5.32</td>
<td>36.70±5.17</td>
</tr>
<tr>
<td>Group II (age above 40)</td>
<td>52.37±7.08</td>
<td>51.89±6.34</td>
</tr>
<tr>
<td>Year of exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I (below 10 Years)</td>
<td>6.70±1.96 (31)</td>
<td>-</td>
</tr>
<tr>
<td>Group II (above 10 Years)</td>
<td>14.44±2.38 (89)</td>
<td>-</td>
</tr>
</tbody>
</table>

SD= standard deviation

Graph 2: Total cholesterol levels of controls and experimental groups

Graph 3: HDL levels of controls and experimental groups
Results of the chromosomal aberration and micronuclei assay are presented in table 3 and graphs 7 and 8. Among the results; significantly elevated (1.30±1.02) levels of minor chromosome aberration were observed in the higher disease duration patients compared to controls and other subjects. Elevated level of MN frequency was found in experimental group II subjects (2.56±0.78) compared to controls.

**Discussion**

The worldwide prevalence of DM had risen spectacularly and the IDF has estimated that 438 million individuals will have diabetes by the year of 2030\textsuperscript{29}. The number of people with type 2 DM is increasing in every country and 75% of people with DM are living in developing countries\textsuperscript{34}. According to the IDF reports in 2019, the prevalence of diabetes in China is 116.4 million, India 77.0 million and America 31.0 million. The male experimental subjects were higher in diabetes levels compared with female subjects. Above the 40 years of age male patients were having a high risk.

Mean blood glucose levels of FBS and HbA1c are the indicators for the development of diabetic complications\textsuperscript{39}. Chronic high blood sugar linked with diabetes may cause damage to blood vessels and nervous strain foremost to obstruct moving many organs and tissues. HbA1c is a very good and sensitive biomarker for diagnosis of type DM2\textsuperscript{28}. 

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**Graph 4: LDL levels of controls and experimental groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Experiment</th>
</tr>
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<tbody>
<tr>
<td>LDL (mg/dL)</td>
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</table>

**Graph 5: VLDL levels of controls and experimental groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Experiment</th>
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<tr>
<td>VLDL (mg/dL)</td>
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</table>
Graph 6: Serum creatinine levels of controls and experimental groups

Graph 7: Chromosomal aberration frequencies of controls and experimental groups

Table 2
Evaluations of the biochemical profiles in diabetes mellitus patients and controls

<table>
<thead>
<tr>
<th>Particulars</th>
<th>No. of Sample</th>
<th>FBS# 100 mg/dL</th>
<th>HbA1c (%) 4% and 5.6%</th>
<th>Total cholesterol 125 to 200 mg/dL</th>
<th>HDL-C 40 mg/dL</th>
<th>LDL-C 100 mg/dL</th>
<th>VLDL-C 2 to 30 mg/dL</th>
<th>Creatinine 0.7 to 1.2 mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiments</strong></td>
<td></td>
<td></td>
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<tr>
<td>Group I (below 40)</td>
<td>31</td>
<td>133.5±16.15</td>
<td>7.5±2.6</td>
<td>189.13±15.52*</td>
<td>44.45±7.56</td>
<td>119.52±21.52*</td>
<td>26.38±3.26</td>
<td>1.21±0.31</td>
</tr>
<tr>
<td>Group II (above 40)</td>
<td>89</td>
<td>156.5±18.04*</td>
<td>9.2±1.9</td>
<td>221.74±39.31*</td>
<td>44.31±6.60</td>
<td>111.24±18.59*</td>
<td>23.64±4.13</td>
<td>1.22±0.42**</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Group I (below 40)</td>
<td>31</td>
<td>85.66±7.11</td>
<td>3.5±0.2</td>
<td>173.74±12.17</td>
<td>48.12±7.86</td>
<td>97.64±4.09</td>
<td>27.06±3.54</td>
<td>1.04±0.19</td>
</tr>
<tr>
<td>Group II (above 40)</td>
<td>89</td>
<td>90.83±5.03</td>
<td>4.1±0.3</td>
<td>166.56±18.96</td>
<td>47.24±6.99</td>
<td>88.24±10.26</td>
<td>24.80±4.64</td>
<td>1.07±0.34</td>
</tr>
</tbody>
</table>

# Fasting blood sugar
*significantly elevated compared to controls subjects and the reference range
**significantly elevated when compared to controls subjects
The study also indicated that both parameters are very important tools for diagnosis and prognosis approach of DM patients. The same results were found by some other investigators as well\textsuperscript{39}. Diabetes is a major metabolic syndrome and it altered the lipid and lipoprotein level\textsuperscript{18}. Basit et al\textsuperscript{4} reported that poor glycemic index will increase the relationship with hypertension and hypertriglyceridemia risk. The total cholesterol and LDL levels were found to be higher in the DM patients. Ozder\textsuperscript{32} reported DM patients having the different level of TG and VLDL. Another study had perceived the prevalence of lipid abnormalities like hypercholesterolaemia, hypertriglyceridaemia, high HDL and low LDL in the T2DM patients in comparison to controls\textsuperscript{15}.

In the present study, it was found out that the total cholesterol level and LDL-C level (lower aged) were significantly elevated in group I DM experimental subjects but HDL-C level was insignificantly decreased when compared to controls and normal in reference range. VLDL-C and creatinine levels were insignificantly different from the controls. Patel et al\textsuperscript{33} studies found out a significant correlation of HbA1c with VLDL and LDL levels.

Bijlani et al\textsuperscript{9,10} reported that HDL-C level has significantly decreased in obese DM patients and they stimulate higher risk for premature coronary artery disease\textsuperscript{19}. In this study it was found out that the women had higher HDL-C level compared to men. The uneven level of triglycerides, total cholesterol, LDL and HDL has caused cardiovascular disease\textsuperscript{27} and it leads to increase morbidity and mortality worldwide\textsuperscript{36}. Hyperglycemia and atherosclerosis are associated with type-II DM\textsuperscript{14}.

Diabetes has increased the production of ROS molecules or damages the antioxidant defense systems, which results in producing oxidative stress to bio-molecules\textsuperscript{6}. DM associated with nucleolar organizing regions leads to be responsible for numerical chromosome anomalies due to non-disjunction process.\textsuperscript{43} The present study pointed out significantly
elevated level of minor chromosome aberration observed in the higher disease duration patients compared to controls and other subjects. MN frequency has significantly elevated in experimental group II subjects compared to controls. Micronuclei frequency and chromosomal aberrations assay are sensitive markers for detection of DNA damage. Increased MN formation is related to hypertension in diabetes and MN frequency is a clinical marker for prediction of diabetic microvascular and macrovascular complications.

Conclusion
The monitoring of biochemical levels and genetic instability like CA and MN frequency in diabetic persons had a competence to be used as biomarker in diabetic complications. This study indicates clinical and cytogenetic alteration. Genetic components play an important role in the pathogenesis of type 2 diabetes.

Acknowledgement
We sincerely acknowledge participants for making this work possible. We extend our sincere thanks to the Authorities of Department of Science and Technology (DST)-Science and Engineering Research Board for providing the project fund (DST/SERB/EEQ/2016/000781) to establish the Genetic laboratory and our sincere thanks go to Department of Zoology, Sri Vasavi College, Erode for supporting and providing necessary infrastructure facilities required for this study.

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(Received 28th April 2022, accepted 01st June 2022)