Study of Magnesium, Chloride, Lipidperoxidation, Lipidhydroperoxides, among Typeii Diabetic Patients with Elevated Fasting Sugar Level

V.N.Janakarajan¹, A. Ambika², KK Thirunavukkarasu³, Celestine rajManohar⁴, M.Rajendran⁵, Chandrabose⁶, SirajFatima⁷, Saravana balaji⁸, M.Karunakaran⁹ and Karmarkar¹⁰

¹Dept of Biochemistry IRT Perundurai Medical College, Erode, Tamil Nadu, INDIA
²Bharathiar University, Coimbatore, Tamil Nadu, India
³Dibetologist Best Diabetic Lab, Gopichettipaayam, Tamil Nadu, INDIA
⁴IRT Perundurai Medical College, Erode, Tamil Nadu, India
⁵Dept of Surgery, IRT Perundurai Medical College, Erode, Tamil Nadu, INDIA
⁶Dept of Physiology, IRT Perundurai Medical College, Erode, Tamil Nadu, INDIA
⁷IRT Perundurai Medical College, Erode, Tamil Nadu, INDIA
⁸RVS dental college sulur Coimbatore, Tamilnadu, INDIA
⁹Dept of Biotechnology, Bharath university Selaipyur, Chennai now adviser Biotechnology, Chennai, Tamil Nadu, INDIA
¹⁰Department of community medicine, All India institute of Medical sciences (AIIMS) New Delhi, INDIA

Abstract

We have compared the level of magnesium, chloride, lipid peroxidation, lipid hydroperoxides among type II diabetic patients with two groups with low (FD1) 154±6.0mg/dL fasting plasma glucose and high (FD2) 273 ±10mg/dL fasting plasma glucose. One hundred known type II diabetic patients and one hundred known healthy subjects were compared. The lipid profile study was carried out using standard biochemical methods. We have found magnesium 0.80±0.20mg/dL, chloride 52±4mmol/L was significantly decreased but also there is a concomitant increase in lipid peroxidation 7.4±0.3µ moles/L and lipid hydroperoxide 5±1.4µ moles/L among FD2 subject with increase in glucose. The total cholesterol, LDL cholesterol, triglycerides were found to be higher among NIDDM subjects.

Keywords: NIDDM- Noninsulin dependent diabetes mellitus, LPO- Lipid peroxidation, LOH-Lipidhydroperoxide, HDL-Highdensity lipoprotein, LDL-Lowdensity lipoprotein, VLDL-Very lowdensity lipoprotein, Tgl-Triglyceride, TC-Totalcholesterol, Mg²⁺- Magnesium, Cl—Chloride.

Introduction

The incidence of diabetes in the world in the year 2000 among adult is 171 million. The international diabetes federation has projected the number of people with diabetes for 2003 is 194 million and in 2025 it will be 334 million¹.

The study made between april 2003 to march 2005 on the incidence of diabetes in india among urban rural area was made by mohan and his co workers under National non-communicable disease (NCD) programme and reported the prevalence of self reported diabetes in india was 11.3% in urban area but in the rural area lowest prevalence of diabetes 0.7% was recorded ².

Recently the incidence of obesity was assessed in several districts of Haryana in north india under WHO guidelines and reported the obesity prevailed in the age group of 18-21 years³.

Diabetes is one of the lifestyle disease, India is called the diabetic capital of the world. The research on trace elements among NIDDM for the past few decades is few and far so it is necessary to carry out the research on trace elements.

Materials and Methods

Description of patients and healthy volunteers
The studies were made on fasting blood samples obtained from the following subjects, one hundred adult patients diagnosed as Non insulin dependent diabetes mellitus (NIDDM) attending regularly as out patients at perundurai Medical college and hospitals, Perundurai and best diabetic lab Gopichettipalayam INDIA 638053. The patients were mostly drivers and daily wages workers enlisted for the study. The study was made over a period of one month. The patients were under the medical supervision of doctors working at perundurai medical college and also at best diabetic hospital Gopi chettipalayam. Erode district Tamilnadu. Blood glucose control, dietary advice monitoring for complications and therapeutic measures were carried out by the medical and paramedical staffs in the centre. Our study includes diabetic patients with varying degrees of severity of complications. The number of years after the onset of disease was recorded.

We have adopted a criteria for diagnosing diabetes on the recommendation of WHO group on diabetes 1985⁴.
The type II diabetic patients were divided into low (FD1) 154±6.0mg/dL and high (FD2) 273±10mg/dL fasting plasma blood glucose. The duration of diabetes for (FD1) subject is 2 to 4 years and for (FD2) it is 4 to 6 years.

**Sample Collection:** Blood was drawn by vein puncture from subjects after 12 hours of overnight fasting. Only 2.5ml of blood was allowed to be taken. Heparin was added as anticoagulant. The plasma was separated from the blood by spinning at 4,000rpm and the following assays were made on blood plasma:

- **a) glucose**
- **b) Urea**
- **c) Creatinine**
- **d) lipid profile**

VLDL cholesterol was measured by using friedwald equation: $\text{VLDL Cholesterol (mg/dL)} = \frac{\text{TGL}}{5} = \text{VLDL cholesterol}$

**HDL cholesterol+ VLDL cholesterol minus total cholesterol = Low density lipoprotein cholesterol**

**Assay of Oxidative damage:** The lipid hydroperoxides in the plasma was measured with the FOX II reagent. The lipid peroxidation product in the plasma was measured by the TBARS method.

**Assay of Trace elements:** Plasma Magnesium was measured by the method of Xylidyl blue. Plasma Chloride was measured by the Mercuric thio cyanate (MTC) method. Throughout the experiment double distilled water was used.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Contents</th>
<th>NIDDM with low level fasting glucose (FD1)</th>
<th>NIDDM with high level fasting glucose (FD2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Number analysed</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>Duration of Diabetes mellitus (Years)</td>
<td>2-4</td>
<td>4-6</td>
</tr>
<tr>
<td>3</td>
<td>Family history of diabetes mellitus %</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>Males %</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>Females %</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>Age (Years)</td>
<td>40±2.6</td>
<td>50±1.6</td>
</tr>
<tr>
<td>7</td>
<td>Height (cm)</td>
<td>150±7.8</td>
<td>154±4.0</td>
</tr>
<tr>
<td>8</td>
<td>Weight (kg)</td>
<td>64±4.0</td>
<td>65±2.0</td>
</tr>
<tr>
<td>9</td>
<td>Smokers(%)</td>
<td>80%</td>
<td>80%</td>
</tr>
<tr>
<td>10</td>
<td>Fasting plasma blood glucose (mg/dL)</td>
<td>154±6.0</td>
<td>273±10</td>
</tr>
<tr>
<td>11</td>
<td>Post Prandial blood glucose (mg/dL)</td>
<td>273±10</td>
<td>360±10</td>
</tr>
<tr>
<td>12</td>
<td>Urea (mg/dL)</td>
<td>31±10</td>
<td>46±10</td>
</tr>
<tr>
<td>13</td>
<td>Creatinine (mg/dL)</td>
<td>1.8±0.25</td>
<td>2.0±0.60</td>
</tr>
<tr>
<td>14</td>
<td>Urine sugar (Number of persons)</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>15</td>
<td>Urine Albumin (Number of persons)</td>
<td>36</td>
<td>60</td>
</tr>
<tr>
<td>16</td>
<td>HbA1c level %</td>
<td>10.5±1.1 %</td>
<td>11.0±1.5 %</td>
</tr>
</tbody>
</table>

NIDDM subjects with low level of fasting plasma blood glucose FD1 compared with FD2 with high level of fasting plasma blood glucose

<table>
<thead>
<tr>
<th>S. No</th>
<th>Contents</th>
<th>Healthy control</th>
<th>NIDDM with low level fasting glucose (FD1)</th>
<th>NIDDM with high level fasting glucose (FD2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Number analysed</td>
<td>100</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>Lipid peroxidation (micro moles/litre)</td>
<td>1.9±0.3</td>
<td>6.0±2.07</td>
<td>7.4±0.3**</td>
</tr>
<tr>
<td>3</td>
<td>Plasma lipid hydroperoxides (micromoles/litre)</td>
<td>3.1±1.0</td>
<td>4.0±1.0</td>
<td>5±1.4***</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD for 100 individual: units are expressed, as – Plasma lipid peroxidation μ moles/L. Plasma lipid hydroperoxides μ moles/L.. Statistical analysis: student “t” test (Significantly different at ** P<0.001)**
Table 3
Distribution of cholesterol (mg/dL) in plasma lipoprotein in normal and NIDDM with FD1 and FD2 subjects studied

<table>
<thead>
<tr>
<th>S.No</th>
<th>Contents</th>
<th>Total cholesterol (Tc) (mg/dL)</th>
<th>HDL cholesterol (mg/dL)</th>
<th>LDL cholesterol (mg/dl)</th>
<th>VLDL cholesterol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Controls</td>
<td>210±19</td>
<td>55±5</td>
<td>105±12</td>
<td>48±12</td>
</tr>
<tr>
<td>2</td>
<td>NIDDM with low fasting glucose (FD1)</td>
<td>214±8</td>
<td>39±5</td>
<td>123±3</td>
<td>53±4</td>
</tr>
<tr>
<td>3</td>
<td>NIDDM with high fasting glucosel (FD2)</td>
<td>218±8</td>
<td>39±1.5</td>
<td>120±3</td>
<td>60±10</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD milligram/dL for total cholesterol (Tc), HDL-High density lipoprotein cholesterol, LDL-low density lipoprotein cholesterol, VLDL—very low density lipoprotein cholesterol.

Table 4
Comparison of Plasma magnesium (mg/dL) level and Chloride (mMol/L) in both FD1 and FD2 with that of the healthy control

<table>
<thead>
<tr>
<th>S.No</th>
<th>Contents</th>
<th>Magnesium (mg/dL)</th>
<th>Chloride (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Controls</td>
<td>1.8±2.6</td>
<td>98±10.9</td>
</tr>
<tr>
<td>2</td>
<td>NIDDM with low level of fasting glucose (FD1)</td>
<td>1.08±0.6</td>
<td>79.36±2.6</td>
</tr>
<tr>
<td>3</td>
<td>NIDDM with high level of fasting glucose (FD2)</td>
<td>0.80±0.20</td>
<td>52±4</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD for 100 individual: units are expressed as—plasma Magnesium milligram/dL, Plasma chloride level mMol/L, Statiscal analysis: Student “t” test (Significantly different at ***P<0.001)

Figure 1
Comparison of Magnesium With Low Level of Fasting Sugar Level (FD1)
Glucose 275±10 mg/dl

Mg 0.80±2.0 mg/dl

Figure-2
Comparsion of magnesium with high level of fasting sugar level fd2 among niddm patient

Glucose 154±6.0 mg/dl
Chloride 79.36±2.6 mg/dl

Figure-3
Comparsion of chloride with low level of diabetic fasting sugar level (fd1)
Results and Discussion

**Table-1:** Gives the general data on the subjects studied and their blood plasma analysed for various biochemical parameters.

The NIDDM subjects were divided into low (FD1) plasma fasting blood glucose 154±6.0 mg/dL, the duration was between 2 to 4 years and high plasma fasting blood glucose (FD2) 273±10mg/dL, the duration was between 4 to 6 years. No gross variation was observed in height and weight measurements in both (FD1) and (FD2). (FD2) subject showed elevation in fasting and postprandial glucose level. Blood plasma urea was found to be elevated in (FD2)46±10mg/dL indicating the severity of renal complications.

**Table-2:** Represents the data on oxidant stress these data were compared with healthy controls. Plasma lipid peroxidation was found to be higher in FD 2 7.4±0.3 μmoles/L but in FD2 the lipid hydroperoxides was found to be 5±1.4μ moles/L when compared with that of FD1. The increase in both lipid peroxidation, lipid hydroperoxide formation is parallel with that of increasing glucose level this is compared in figure 1 and 2. The presence of glucose and albumin in urine indicates the severity of renal complications.

**Table-3:** Shows the distribution of cholesterol (mg/dL) in plasma lipoprotein fractions in FD1, FD2 that is matched with that of controls in fasting plasma blood samples. Low density lipoprotein cholesterol was found to be higher among NIDDM subjects and high density lipoprotein cholesterol was found to be lowered.

**Table-4:** Gives the comparison of plasma magnesium(Mg$^{2+}$) and Chloride level (Cl$^{-}$) in NIDDM when compared with that of healthy controls. The level of magnesium was found to be severely decreased among NIDDM subjects in (FD 1) it is 1.08±0.6 mg/dL magnesium but in (FD 2) it is 0.80±0.20mg/dL magnesium. There is a moderate reduction in chloride value was observed in FD 1 subject 79.36±2.6 mMol/L but in FD 2 it is significantly reduced 52±4.0 mmol/L chloride. In our observation both magnesium and chloride showed inverse relation with fasting plasma glucose level. This is compared in figure 3 and 4.

**Discussions:** The glycaemic control in the fasting subjects in both FD1 (154mg/dL) and in FD2 it is (275mg/dL) is very poor as can be seen from high glucose level.

The post prandial plasma glucose in (FD 1) is 273±2.0 mg/dL plasma but in FD 2 it is 360mg/dL plasma. The glycated haemoglobin (HbA$_1C$) in FD1 was10.5±1.1% but in FD2 it was found to be 11.0±1.5% when compared to healthy control. This indicates the patients were in poor glycaemic control.

**Status of Lipid peroxidation and Lipid hydroperoxide:** In our observation the lipid peroxidation was increased to more than five fold among NIDDM in FD1 subjects however in FD2 LPO is elevated to more than six fold, like wise lipid hydroperoxide formation was found to be significantly increased among FD2 subject. The increase in both lipid peroxidation and lipid hydroperoxide formation confirms oxidative stress.

Oxidative stress can impair insulin action with a change in the physical state of plasma membrane of target cell. Elevated levels of circulating peroxides can cause damage to islets of langerhans resulting in cell damage and loss of function. The elevation in glucose level in our observation might be due to an increase in the level of free radicals in terms of lipid peroxidation and lipid hydroperoxide formation.
Status of Magnesium and Chloride: Magnesium serves as a Cofactor for more than 300 enzymatic reactions. Its deficiency correlate well with a number of chronic cardiovascular disease including hypertension, diabetes mellitus, hyperlipidaemia. Intracellular magnesium concentration plays a pivotal role in modulating insulin mediated glucose disposal. It was proved experimentally that daily magnesium administration contributes to improve insulin action to metabolise glucose.

In our observation plasma magnesium was significantly decreased among FD2 subjects and in FD1 subjects. We have observed inverse magnesium value with high fasting glucose level among NIDDM.

Chloride dominates 70% of the total body’s negative ion of the blood. The chloride was found to be significantly reduced among FD2 subjects and moderately decreased in FD1. Kikuchi and his coworkers have already reported that plasma chloride concentrarion was found to be decreased with impaired insulin action.

Rodriguez and his coworkers have proved experimentally that oxidative stress can promote sodium chloride retention by the kidneys.

The total body chloride depletion was reported under four major conditions: a) inadequate sodium chloride intake b) Interstitial nephritis c) Hyperglycaemia d) Nephrotic syndrome.

We have observed decrease in chloride level with increase in fasting glucose value among subjects among FD2 subject.

Conclusion
We have observed low level of Magnesium and Chloride among type II diabetic patients with increase in fasting uncontrolled glucose level. The decrease in Magnesium and Chloride might be due to a) renal impairment with prolong glucosuria 2) autoxidation of glucose which generates free radicals in terms of lipidperoxidation and lipid hydroperoxide in turn which damages the integrity of cell membrane in uncontrolled diabetic patient.

The research on trace elements has to be carried out to a greater extent, the depletion in Magnesium and chloride with increase in lipidperoxidation and lipid hydroperoxide is a real threat to the humans who were suffering from Non insulindependent diabetes mellitus.

References