Levels of Total Antioxidant Capacity (TAC) and oxidative stress in type 2 diabetes mellitus in Kanpur region

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Abstract: Background: Hyperglycemia is associated with decreased antioxidant capacity and increased oxidative stress. Micro and Macro vascular complications are mainly caused by oxidative stress in Diabetes Mellitus. The total antioxidant capacity assay is used to determine total antioxidant power of plasma. Objective: To find levels of Total antioxidant capacity, oxidative stress and Lipid profile in Type 2 Diabetic patients. Methods: We assessed Total Antioxidant Capacity (TAC), Malondialdehyde (MDA) and Lipid Profile in 120 patients of type 2 diabetes Mellitus and 120 healthy controls. This study was conducted in Rama Medical College, Hospital & Research centre, Kanpur. Student t test was used for statistical analysis. Result: The study showed that decreased total antioxidant capacity, increased Malondialdehyde levels and altered Lipid profile in type 2 diabetics compared with healthy controls. Conclusion: Total Antioxidant Capacity (TAC) significantly decreased in Type 2 DM compared to control group.

Keywords: Total antioxidant capacity, Oxidative stress, Malondialdehyde, Diabetes mellitus

Introduction
Diabetes mellitus is a chronic metabolic disorder characterized by impaired glucose and lipids metabolism due to defect in insulin secretion (beta cell dysfunction) or action (insulin resistance) or both [1]. Which may affect all age groups and sex [2]. Changes in human behaviour and lifestyle over the last century have resulted in an increase in the incidence of Diabetes worldwide [3].

Long term complications of diabetes are diabetic nephropathy, neuropathy, retinopathy and cardiovascular diseases, which bring high cost for both individual and society due to the development of complications affecting the vascular system, kidney, retina and peripheral nerve in diabetic patients [4-6]. Worldwide 415 million people are diabetic and it is expected to rise to >642 million by 2040. The Indian statistics showed prevalence is 8.7 and 69.2 million Diabetic cases in adult population [7]. This is according to IDF diabetes atlas 7th edition-2015 update. Oxidative stress is Oxidative damage of cell, tissues or organs by free radicals [8-9].

Oxidative stress plays a major part in diabetic progression [10]. In pathogenesis of diabetes and relationship between oxidative stress and complications, free radicals play major role [11-12].

Non enzymatic protein glycation, glucose auto oxidation and activation of polyol path way with increased oxidative stress is the possible cause of long term and high blood glucose levels in uncontrolled diabetes mellitus [13-14]. A way of assessing the antioxidant levels of plasma recently proposed as TAC. The total radical trapping antioxidant parameter (TRAP) also used as TAC or TAS [15].

The antioxidant capacity of all the antioxidants present in serum/plasma, foods (dietary total antioxidant capacity) and other body fluids considered as TAC [16-18]. TAC gives cumulative parameter instead of simple sum of measurable antioxidants [2]. Total antioxidant capacity measurement is useful indicator of risk associated with activity of free radicals in type 2 diabetics [19]. Glucose
attached non-enzymatically to hemoglobin in RBC throughout 90 days of its life time. HbA\textsubscript{1c} gives previous three months average blood glucose levels [20].

Higher levels of glycated hemoglobin seen in individuals with high levels of blood glucose. The formation of HbA\textsubscript{1c} is dependent on ambient glucose concentration. About 3-6% of HbA is glycated in normal persons. Depending on the degree of hyper glycemia the percentage may be double or triple in diabetics [21]. It is now believed that the reduction of glycated hemoglobin to less than 7% is the aim of antidiabetic treatment [20].

**Material and Methods**

The study was conducted in the Department of Biochemistry with the collaboration of Department of General Medicine in Rama Medical College, Hospital and Research Centre, Kanpur. 120 patients with type 2 diabetes and 120 healthy controls were included in the study.

**Group I: Control group**

a) This group consists of age and sex matched non-diabetic healthy subjects.

b) Age group between 30-60 years from both sex.

**Group-II: Study group**

**Inclusion criteria:**

a) Type 2 Diabetes Mellitus

b) Subjects with age group of 30-60years from both sex

c) Duration of Diabetes Mellitus is 5 years or more

**Exclusion criteria:**

a) Patients with type I Diabetes Mellitus

b) Pregnant and lactating females

c) Patients taking diuretics and lipid lowering drugs.

d) Patients with smoking, liver disease, thyroid disease, tuberculosis, cancer patients and acute or chronic inflammatory diseases.

Sample Collection: Five ml of venous blood was collected using aseptic precautions in fasting state into a plain and EDTA vials from study subjects. Then the samples were centrifuged for 15 minutes at 3000 rpm. The separated plasma (fluoride vial) and serum (plain vial) were stored until further analysis at –50°C.

**Determination of Biochemical parameters:**

Glucose and Lipid profile were estimated on fully automated analyzer. Malondialdehyde (MDA) was estimated as thiobarbituric acid reactive substances (TBARS). HbA1c was estimated by Ion exchange resin method. Total antioxidant capacity was analyzed by ferric reducing ability of plasma (FRAP).

**Statistical Analysis:** Results were expressed as mean ± standard deviation. The significance of difference in means between controls and study group was done by unpaired ‘t’ test.

**Results**

Data analysis was done using SPSS programme. p value was used to compare the groups. p value < 0.05 was considered significant. Table-1 shows that MDA levels were significantly higher in the diabetes mellitus compared with control group. The mean TAC levels were significantly lower in the diabetes mellitus compared with control group.

TC, TG, HbA\textsubscript{1c} levels were significantly higher in the diabetes mellitus compared with control group. HDL-C levels were significantly lower in the diabetes mellitus compared with control group. LDL-C levels were higher in the diabetes mellitus compared with control group.

**Fig-1:** Comparison of TAC, MDA between Control and Study group.
Table-1: Comparison of study variables in subjects with diabetes mellitus and controls

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameters</th>
<th>DM (n=120) Mean ± SD</th>
<th>Controls (n=120) Mean ± SD</th>
<th>‘p’ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>FBS</td>
<td>172±16.43</td>
<td>86.48±5.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2.</td>
<td>PPBS</td>
<td>236.31±42.56</td>
<td>108.2±5.69</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3.</td>
<td>HbA1c</td>
<td>8.47±0.64%</td>
<td>6.31±1.3%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>4.</td>
<td>TC</td>
<td>176.9±36.09</td>
<td>144.9±34.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>5.</td>
<td>TG</td>
<td>137.9±59.05</td>
<td>111.0±29.83</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6.</td>
<td>HDL-C</td>
<td>44.8±15.77</td>
<td>51.8±10.53</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>7.</td>
<td>LDL-C</td>
<td>151.2±64.03</td>
<td>117.7±26.24</td>
<td>&lt;0.0753</td>
</tr>
<tr>
<td>8.</td>
<td>MDA</td>
<td>7.2±1.06</td>
<td>3.76±0.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>9.</td>
<td>TAC</td>
<td>0.6±0.09</td>
<td>1.43±0.15</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

FBS-fasting blood sugar, PPBS- postprandial blood sugar, HbA1c-glycated hemoglobin, TC-total cholesterol, TG-triglycerides, HDL-C – high density lipoprotein cholesterol, LDL-C – low density lipoprotein cholesterol, MDA-malondialdehyde, TAC-total antioxidant capacity

Discussion

The mean MDA levels were increased in type 2 Diabetes Mellitus, comparing with controls. Changes in the tissue content and activity of antioxidant defense system occurs as increased oxidative stress in diabetes, also due to oxygen free radical generation, non enzymatic glycation, auto oxidation of glycation end products [22-23]. Decomposition of Poly Unsaturated Fatty Acids (PUFA) produces MDA, which is a stable end product of lipid peroxidation. There are several studies supporting the theory of increased oxidative stress in diabetes mellitus by way of estimating MDA. Sai Ravi Kiran from Pondicherry have found a marked increase in MDA levels in diabetic patients with compared to healthy controls [24]. Chavan have also observed similar results among a study population from Gujarat [25].

TAC is significantly decreased in diabetic patients compared with control group. TAC is also known as Total radical trapping antioxidant parameter (TRAP). Existence of low levels of circulating antioxidants in diabetic patients suggestive of decreased TAC. O.M. Akinosun have found a marked decrease in TAC levels in diabetic patients with compared to control group [19]. In this study TAC levels were decreased may be due to:

a) As a result of Diabetes Mellitus excess free radicals are produced which are neutralized by antioxidants [26].

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b) Auto oxidation of glucose and non enzymatic glycation in diabetes mellitus most likely increased sources of free radicals [27].

c) Poor socioeconomic status of an individual leads to decreased intake of dietary antioxidants such as Vitamin C, Vitamin E, Beta carotene and sulphur containing amino acids like methionine.

d) Lower available state of micro nutrients like selenium, zinc, copper, manganese causes insufficient synthesis of antioxidant enzymes such as super oxide dismutase and glutathione peroxidise [28].

Alterations in lipid profile were observed in diabetes with compared to control group. It is due to improper functioning of insulin which affect liver apo lipoprotein production which in turn regulation enzymatic activity of lipoprotein lipase and cholesterol transport protein causes dyslipidemia in type2 diabetes [29].

Conclusions

It is well known that diabetes is accompanied by pronounced oxidative stress. Oxidative damage can cause an imbalance in the activity of antioxidant enzymes it leads to progression of diabetes and its complications. This study suggests that estimation of antioxidant levels may be useful in the prevention of the diabetic complications. Supplementation of antioxidant rich components can prevents progression of diabetes and its complications. Future studies are required to know the mechanism responsible for the oxidative stress in development and progression of complications in diabetes.

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Conflicts of interest: There are no conflicts of interest.

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