Serum Adiponectin - A Risk Factor for Diabetes Mellitus.

Bhargavi SK\(^1\)*, BV Maruthi Prasad\(^2\), HL Vishwanath\(^2\).

\(^1\)Department of Biochemistry, Sri Siddhartha Medical College & Research Centre, Tumkur, Karnataka, India.
\(^2\)Department of Biochemistry, Bangalore Medical College & Research Institute, Bangalore, Karnataka, India.

ABSTRACT

Diabetes mellitus (DM) is a group of metabolic disorders of carbohydrate metabolism in which glucose is underused, producing hyperglycemia. An epidemic of Type 2 DM is underway and has become one of the world’s most important public health problems. Adiponectin, a protein hormone, is produced by the adipose tissue. It has pronounced effects on the metabolism of both carbohydrates and lipids in liver and muscle. It is considered to have anti-inflammatory and anti-atherogenic effects. Levels are low in presence of insulin resistance and show important relationships with development of type 2 Diabetes Mellitus and its complications. Hence serum adiponectin levels were measured as a risk factor for DM. A case control study was done to estimate serum adiponectin levels in 30 type 2 DM patients and compare with age and sex matched controls and to assess adiponectin levels as an early predictor of onset of type 2 DM. Patients with renal, liver and thyroid disorders were excluded. Serum adiponectin was measured by competitive ELISA method. The mean serum adiponectin levels in cases is 5.91±1.47μg/mL and p value <0.001. The mean serum adiponectin levels in controls is 11.40±1.88μg/mL and p value <0.001. The mean serum adiponectin levels in diabetics without complications is 6.67±1.01μg/mL and p value of <0.001**. Diabetic patients have lower levels of adiponectin compared to healthy controls. Results of this study also show that diabetic patients with complications have lower adiponectin levels compared to that of diabetics without complications. Thus adiponectin may represent a link between insulin resistance and endothelial dysfunction making it an attractive candidate marker for further studies in type 2 DM.

Keywords: Adiponectin; Adipose tissue; Diabetes Mellitus; Dyslipidaemia
INTRODUCTION

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underused, producing hyperglycemia [1]. It is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both [2]. With an increasing incidence worldwide, DM will be a leading cause of morbidity and mortality for the foreseeable future [3].

Several distinct types of DM are caused by a complex interaction of genetic and environmental factors. When fully expressed, diabetes is characterized by fasting hyperglycemia. Hyperglycemia sufficient to cause pathologic functional changes may quite often be present for a long time before the diagnosis is made. The effects of DM include long term damage, dysfunction and failure of various organs, especially eyes, kidneys, heart and blood vessels.

An epidemic of Type 2 DM is underway in both developed and developing countries [4]. It has become one of the world’s most important public health problems. Type 2 DM accounts for 85-90% of diabetes worldwide [5]. It is most commonly diagnosed in those over 40 years of age and the incidence rises to a peak at 60-65 years [5]. However, much younger people are now presenting with type 2 DM, following the rapid rise in childhood obesity.

Traditionally adipocytes have been viewed as energy deposits that store TAG during feeding and release FA during fasting to provide fuel for other tissues. However adipose tissue secretes numerous proteins that have important physiological function eg. Adiponectin, Leptin, Resistin, angiotensinogen, Estrogen, Visfatin etc. These factors participate in autocrine and paracrine regulation within adipose tissue and can affect the function of distant organs such as muscles, pancreas, liver and central nervous system.

Adiponectin is a protein hormone made of 224 aminoacids produced by the adipose tissue. It has pronounced effects on the metabolism of both carbohydrates and lipids in liver and muscle promoting uptake and oxidation of fatty acids by myocytes but blocks the synthesis of fatty acids and gluconeogenesis by hepatocytes. It is considered to have anti-inflammatory and anti-atherogenic effects. Its serum concentration is inversely associated with adiposity. It has direct insulin sensitizing activity. Levels are also low in presence of insulin resistance and show important relationships with development of type 2 Diabetes Mellitus and its complications. Adiponectin may represent a link between insulin resistance and endothelial dysfunction making it an attractive candidate marker for further study in type 2 DM and atherosclerosis. Laboratory assessment of serum adiponectin can be done by ELISA and Radioimmunoassay method.

MATERIALS AND METHODS

Source of data

The study was approved by the ethical and research committee of BMC & RI. It was conducted on patients with type 2 DM attending the outpatient & inpatient Departments of
Medicine in Victoria hospital and Bowring and Lady Curzon Hospitals attached to Bangalore Medical College and Research Institute, Bangalore. The patients and the controls voluntarily participated in this study. All patients with type 2 Diabetes Mellitus aged 40-70 years diagnosed according to American Diabetes Association criteria (FBS ≥126 mg/dl & 2 hour PPBS ≥ 200 mg/dl) and patients already on treatment for DM were included in the study. Patients with history of myocardial infarction, angina, liver, kidney and thyroid diseases which are known to influence serum levels of adiponectin were excluded from the study. 30 age and sex matched healthy individuals were taken as controls. Informed consent was taken from both groups.

Fasting blood samples were collected with all aseptic precautions. 5 ml of blood was collected from mediancubital vein. It was allowed to clot for 30 minutes in a clean dry test tube and was subjected to centrifugation for 20 minutes to separate the serum. The serum samples were stored at -70°C till they were analyzed. ELISA kit provided by Biovendor, Germany, was used for the quantitative measurement of human adiponectin based on competitive enzyme immunoassay.

**Statistical Methods**

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number(%). Significance is assessed at 5% level of significance. Student ‘t’ test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (inter group analysis) on metric parameters. Leven1s test for homogeneity of variance has been performed to assess the homogeneity of variance. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

Significant figures
+ Suggestive significance (P value: 0.05<P<0.10)
* Moderately significant ( P value:0.01<P ≤ 0.05)
** Strongly significant (P value : P≤0.01)

**RESULTS**

In both cases and controls, 36.6% were males and 63.3% were females. In both the study groups, 50% were in the age group 40-50 years, 40% were in the age group 50-60 and 10% were in the age group 61-70 years as per Table 1 and Graph 1. Cases and controls were appropriately age and sex matched as shown in Table 2 and Graph 2.
Table 1: Age distribution of cases and controls

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Cases</th>
<th></th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>40-50</td>
<td>15</td>
<td>50.00</td>
<td>15</td>
<td>50.00</td>
</tr>
<tr>
<td>51-60</td>
<td>12</td>
<td>40.00</td>
<td>12</td>
<td>40.00</td>
</tr>
<tr>
<td>61-70</td>
<td>3</td>
<td>10.00</td>
<td>3</td>
<td>10.00</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.00</td>
<td>30</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Mean ± SD: 52.70±8.47, 52.37±8.77

Samples are age matched with p=0.881

Graph 1: Comparison of cases and controls according to age groups

Table 2: Gender distribution among cases and controls

<table>
<thead>
<tr>
<th>Gender</th>
<th>Cases</th>
<th></th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>36.67</td>
<td>11</td>
<td>36.67</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>63.33</td>
<td>19</td>
<td>63.33</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.00</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

Samples are gender matched with p=1.000
Graph 2: Comparison of cases and controls according to sex

DISTRIBUTION OF SUGAR PARAMETER IN CASES AND CONTROLS

Fasting Blood Sugar

In the 30 cases that were studied, the mean FBS level was 156 mg/dL with a SD of 49.20 mg/dL. The controls had a mean FBS level of 81.87 mg/dL with a SD of 12.16 mg/dL and p value <0.001 as depicted in Table 3 and Graph 3.

Table 3: Comparison of Glucose parameters in cases and controls

<table>
<thead>
<tr>
<th>Glucose parameters</th>
<th>Cases</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (mg/dL)</td>
<td>156.77±49.20</td>
<td>81.87±12.16</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>PPBS (mg/dL)</td>
<td>250.97±97.76</td>
<td>134.13±13.77</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.96±1.98</td>
<td>5.11±0.22</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Graph 3: FBS IN CASES AND CONTROLS
Post Prandial Blood Sugar

In the 30 cases that were studied, the mean PPBS level was 250.97 mg/dL with a SD of 97.76 mg/dL. The controls had a mean PPBS level of 134.13 mg/dL with a SD of 13.77 mg/dL and p value <0.001 as depicted in Table 3 and Graph 4.

Graph 4: PPBS IN CASES AND CONTROLS

HbA1c

The mean HbA1c in the 30 cases was 8.96% with a SD of 1.98%. Mean HbA1c in controls was 5.11% with a SD of 0.22% and p value <0.001 as shown in Table 3 and Graph 5.

Graph 5: HbA1c IN CASES AND CONTROLS

Adiponectin

Normal Serum Adiponectin level is 8-13 µg/ml.
As evident from Table 4 and Graph 6, the mean serum adiponectin levels in cases is 5.91 μg/mL with a SD of 1.47 μg/mL and p value <0.001. The mean serum adiponectin levels in controls is 11.40 μg/mL with SD of 1.88 μg/mL and p value <0.001.

<table>
<thead>
<tr>
<th>Table 4: Comparison of Adiponectin in the two groups studied</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases</strong></td>
</tr>
<tr>
<td>Adiponectin (μg/mL)</td>
</tr>
</tbody>
</table>

Graph 6: Comparison of Adiponectin in the two groups studied

As evident from Table 5 and Graph 7, the mean serum adiponectin levels in diabetics without complications is 6.67 μg/mL with a SD of 1.01 μg/mL and that of diabetics with complications is 4.14 μg/mL with a SD of 0.66 μg/mL and p value of <0.001**.

<table>
<thead>
<tr>
<th>Table 5: Comparison of serum adiponectin in diabetics with and without complications in the cases studied</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters</strong></td>
</tr>
<tr>
<td>S. Adiponectin (μg/mL)</td>
</tr>
</tbody>
</table>

Graph 7: Comparison of serum adiponectin in diabetics with and without complications in the cases studied
From Table 6 it can be seen that LFT, RFT and TSH were in the normal range.

**Table 6 : Comparison of Biochemical parameters in the two groups studied**

<table>
<thead>
<tr>
<th>Bio-chemical parameters</th>
<th>Cases</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dL)</td>
<td>23.33±11.19</td>
<td>22.10±4.99</td>
<td>0.615</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.82±0.28</td>
<td>0.82±0.14</td>
<td>0.991</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dL)</td>
<td>0.52±0.25</td>
<td>0.55±0.26</td>
<td>0.664</td>
</tr>
<tr>
<td>Direct Bilirubin (mg/dL)</td>
<td>0.08±0.075</td>
<td>0.070±0.06</td>
<td>0.583</td>
</tr>
<tr>
<td>Total protein (mg/dL)</td>
<td>7.12±0.67</td>
<td>7.44±0.36</td>
<td>0.026*</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>3.69±0.55</td>
<td>4.04±0.30</td>
<td>0.004**</td>
</tr>
<tr>
<td>Globulin (mg/dL)</td>
<td>3.41±0.53</td>
<td>3.40±0.43</td>
<td>0.936</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>99.47±35.77</td>
<td>25.87±8.31</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>27.60±26.32</td>
<td>20.60±9.70</td>
<td>0.177</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>31.77±26.08</td>
<td>89.30±25.54</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>TSH (μIU/mL)</td>
<td>2.26±1.27</td>
<td>1.87±1.15</td>
<td>0.226</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Several distinct types of DM are caused by a complex interaction of genetic and environmental factors. Depending on the etiology of DM, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization and increased glucose production. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual and the health care system. The function of adipose tissue as an endocrine organ has important implications for understanding the pathophysiologic relationship between excess body fat and pathologic state such as insulin resistance and type 2 DM.

When the vascular endothelium is injured, adiponectin accumulates in the subintimal space of the arterial wall through its interaction with collagens in the vascular intima [6,7]. Adiponectin attenuates TNF-α–induced expression of adhesion molecules in endothelial cells [8], which is an initial step of atherosclerosis. It interferes with TNF-α signaling in endothelial cells. It may have a role in protection against vascular damage [6]. It suppresses the attachment of monocytes to endothelial cell surface, via inhibition of nuclear factor-kappa-B signaling [9], which is an early event in atherosclerotic vascular change. Accumulation of adiponectin in atherosclerotic vascular walls may accelerate its half-life in plasma.
A diabetes-susceptibility locus has been mapped to human chromosome 3q27, where the adiponectin gene is located. Genetic and functional data suggest that adiponectin could be involved in the pathogenesis of type 2 DM [10]. Decreased plasma adiponectin may play a causative role in the development of insulin resistance [6]. It has a substantial role in the pathogenesis of type 2 DM, and could be used as an indicator of risk in addition to the established risk parameters such as obesity and physical activity [10,11]. Chronic insulin resistance in type 2 DM may be related to decreased plasma adiponectin [6].

Clinically normal women have higher adiponectin levels than do men [6]. Sex hormones, including estrogen, progesterone, and androgen may affect the plasma adiponectin level. Adiponectin could be an indicator of macroangiopathy associated with DM. A lower adiponectin level may increase the risk of atherosclerosis. Reduction of BMI results in elevation of the plasma adiponectin [6]. Low concentrations of adiponectin have also been implicated in severe insulin resistance [11-14] in both animal models and humans [15,16]. Low adiponectin concentration is predictive of prospective DM [11,17-19]. Adiponectin dysregulation commences before the onset of DM1[15,20]. Therapy with adiponectin may be advantageous in reversing insulin resistance in lipodystrophic disorders and metabolic syndrome [15,19].

Low concentrations of circulating adiponectin are associated with low high-density lipoprotein cholesterol (HDL-C), obesity, hypertension, and glucose intolerance, all features of the insulin resistance syndrome [21,22]. Adiponectin may represent a link between insulin resistance and endothelial dysfunction making it an attractive candidate marker for further study in T2DM and atherosclerosis [21].

Dr Joachim Spranger et al [10] found that adiponectin concentrations in plasma were lower among individuals who later developed type 2 diabetes than among controls (mean 5·34 μg/mL *SD 3·49+ vs 6·87 μg/mL *4·58+, p<0·0001) and concluded that high concentrations of adiponectin is independently associated with a substantially reduced relative risk of type 2 diabetes in apparently healthy individuals.

In a study done by Chamukuttan Snehalatha et al [11], the mean baseline adiponectin level was lower in the diabetic subjects than in the nondiabetic subjects (11.3 ± 5.5 vs. 16.7 ± 7.6 μg/mL, p = 0.0017). They concluded that low adiponectin level was a strong predictor of future development of diabetes, and HbA1c also showed a positive predictive association.

This is in accordance to the studies done by Helen C Looker et al [23], Mi-Jin et al [24], Bruce B Duncan et al [25] who measured serum adiponectin and found that it was lowest in those with type 2 DM and highest in those with lower normal glucose tolerance and demonstrated a graded inverse association between adiponectin levels and risk of diabetes.

In this study, a subset of diabetic patients with complications like diabetic retinopathy, diabetic cataract and diabetic gangrene were taken. Patients with the other complications such as CAD, CHD, CVD and nephropathy were not included since these diseases are known to influence serum adiponectin levels due to other causes.
The mean serum adiponectin levels in diabetics without complications is 6.67 μg/mL with a SD of 1.01 μg/mL and that of diabetics with complications is 4.14 μg/mL with a SD of 0.66 μg/mL with a p value of <0.001**.

This is in agreement with the studies done by Kikuko Hotta et al [6], Wolfgang Koenig et al [21], Kumada et al [25], Mark P Hamilton et al [26] who showed that levels of adiponectin were low in diabetic patients with complications like CHD and CAD.

Wolfgang Koenig et al [21] in their study observed that the highest risk for type 2 DM as well as acute coronary events was observed in men with low adiponectin in combination with low HDL-C. He suggested that the association between low adiponectin and CHD may be mediated, in part, by the effects of adiponectin on HDL cholesterol. The effects of both low adiponectin and low HDL-C on endothelial dysfunction and their enhancement of an inflammatory response may represent plausible arguments for their additive effect on risk. Hence it can be postulated that low levels of adiponectin in diabetics is a predictor of complications associated with DM.

Insulin regulates the secretion of various proteins from adipose tissue. Elevated plasma insulin in the diabetic subjects in this study may have been responsible for the decreased plasma adiponectin concentrations. Low plasma adiponectin concentration is associated with a decrease in whole-body insulin sensitivity in humans [12-14] and has been shown to be predictive of future development of diabetes in a few studies [17-19]. In addition, high adiponectin concentrations are associated with a reduced risk of type 2 diabetes [19].

It can therefore be speculated that adiponectin, or drugs that stimulate adiponectin secretion or action, could play a role in combination with insulin resistance; mainly type 2 diabetes mellitus and metabolic syndrome [15]. Thiazolidinediones, a class of insulin-sensitizing antidiabetic drugs, increase adiponectin in insulin-resistant patients.

Limitations of the study were small sample size and cross sectional study. A prospective study, over larger time duration, involving follow up of healthy patients who go on to develop DM would have been more precise.

CONCLUSION

The results of the study show that diabetic patients have lower levels of adiponectin compared to healthy controls. This indicates that adiponectin dysregulation commences before the onset of diabetes and it may have a substantial role in the pathogenesis of type 2 DM. It may be an important factor in prediabetes which involves declined insulin sensitivity. Our results suggest that it could be used as an indicator of risk in addition to the established risk parameters such as obesity and physical activity.

Results of this study also show that diabetic patients with complications have lower adiponectin levels compared to that of diabetics without complications. Thus adiponectin may represent a link between insulin resistance and endothelial dysfunction making it an
attractive candidate marker for further studies in type 2 DM and its complications like CAD, CHD and CVD.

It is interesting to note that this parameter can be modified through pharmaceutical and lifestyle interventions, which can be used as preventive measures in reducing the incidence of type 2 DM and its dreaded complications in our country which is the “Diabetes capital of the world.” Adiponectin can also be used as a potential drug target.

REFERENCES


