Original Research Paper

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# ASSOCIATION BETWEEN SERUM ADIPONECTIN LEVELS AND CORONARY ARTERY DISEASE IN TYPE 2 DIABETES MELLITUS PATIENTS

Biochemistry

**KEYWORDS:** Adiponectin, Coronary artery disease, Type 2 diabetes mellitus

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## ABSTRACT

Patients with Type 2 Diabetes mellitus show a 2- to 5- fold increase in coronary heart disease –CAD. Serum adiponectin level is negatively associated with visceral fat and insulin resistance. Its level is decreased in obese, diabetic subjects and subjects with cardiovascular disease. The aim of the study is to assess the association between serum adiponectin levels and coronary artery disease in Type 2 diabetes mellitus subjects. The study was carried out in a total of one hundred and sixty subjects which included one hundred and ten Type 2 diabetes mellitus subjects and fifty controls. The results showed serum adiponectin level was decreased in Type 2 diabetes mellitus subjects when compared with controls. In Type 2 diabetes mellitus subjects with coronary artery disease, the serum adiponectin level was decreased when compared with those without coronary artery disease.

## INTRODUCTION

Diabetes mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycemia[1]. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production[1]. Patients with Type 2 DM show a 2- to 5- fold increase in coronary heart disease –CAD[2]. Serum adiponectin level is negatively associated with visceral fat [3] and insulin resistance [3]. Its level is decreased in obese [4], in diabetic subjects [18] and in subjects with cardiovascular disease [5].

## ADIPONECTIN

### ADIPOSE TISSUE AS AN ENDOCRINE ORGAN

Adipose tissue participates in the regulation of energy homeostasis as an important endocrine organ that secretes a number of biologically active adipokines such as free fatty acid, adipsin, leptin, plasminogen activator inhibitor-1, resistin, and TNF- [6]. Adiponectin is one such adipokine.

### ADIPONECTIN AND INSULIN RESISTANCE

Adiponectin appears to modulate insulin sensitivity by stimulating glucose utilization and fatty acid oxidation via the phosphorylation and activation of AMPK in both muscle and liver cells [7,8].

### ADIPONECTIN AND ATHEROSCLEROSIS

Adiponectin protects against all stages of atherosclerotic plague formation by the following mechanism [9]

1) Stage of endothelial dysfunction:
Protective action of adiponectin –

- Increase NO & eNOS (endothelial NO synthase)
- Decreases endothelial cell apoptosis & reactive oxygen species formation

2) Stage of plague initiation and progression:
Protective action of adiponectin –

- Decreases ICAM-1, VCAM-1, E-Selectin, TNF- , IL-8, foam cell formation, LDL oxidation, smooth muscle cell (SMC) proliferation and migration.
- Increases IL-10

3) Stage of plague rupture and thrombosis:
Protective action of adiponectin –

- Increases tissue inhibitor of metalloproteinase – 1 (TIMP-1)
- Decreases fibrous cap thinning, platelet aggregation and thrombus formation.

### ADIPONECTIN AND OBESITY

Plasma adiponectin levels were decreased in obesity. This reduction may play a causal role in the development of insulin resistance. Obesity also decreased the expression levels of adiponectin receptors, thereby reducing adiponectin sensitivity, which finally leads to insulin resistance [10].

## AIM OF THE STUDY

The aim of the study is to assess the association between serum adiponectin levels and coronary artery disease (CAD) in Type 2 diabetes mellitus subjects.

## MATERIALS AND METHODS

### STUDY POPULATION:

The study was carried out in a total of one hundred and sixty subjects which included one hundred and ten Type 2 diabetes mellitus subjects and fifty controls.

The diabetes mellitus subjects were further categorised as those without coronary artery disease (normal ECG/ treadmill test negative) n = 48 (group 1) and those with coronary artery disease (coronary angiogram proven cases) n = 62 (group 2). The mean age of the diabetes mellitus group was 50.8 years.

For the control group (group 0), age and sex matched apparently healthy subjects. The mean age of the control was 49.4 years.

### INCLUSION CRITERIA:

The minimum duration of diabetes was 5 years in all Type 2 diabetes mellitus subjects. The subjects were on oral hypoglycaemic treatment.

### EXCLUSION CRITERIA:

Subjects who were on treatment with insulin and/ or thiazolidinediones were excluded from the study.

Subjects who had liver disorders, hepatitis, cirrhosis, renal failure, inflammatory disorder or malignancy were excluded from the study.

### PROTOCOL OF STUDY:

The subjects were clinically examined and height and weight measurement were recorded. Blood pressure was also recorded. The body mass index was calculated from the height and weight measurement using the formula

$$\text{Body mass index} = \frac{\text{Weight in kilograms}}{\text{Height in metre}^2}$$

Fasting and 2-hours post prandial blood samples were collected. Plasma was separated from fasting and post-prandial blood samples collected in sodium fluoride – potassium oxalate tubes and was used for estimating plasma glucose immediately. Serum was separated from fasting blood samples collected in the plain tubes and divided into two parts. One part of the serum was used for estimating total

cholesterol, HDL cholesterol and triglycerides immediately. The other part of the serum was stored at –20ºC and was used for estimation of adiponectin and insulin by ELISA method. The ELISA procedure was carried out within 30 days of sample collection.

**ESTIMATION OF SERUM ADIPONECTIN**

**Methodology:**

Enzyme linked immunosorbent assay method using Avibion Human Adiponectin (Acrp30) ELISA Kit from Orgenium Laboratories.

**Instrument:**

Triturus analyzer – an open system automatic ELISA analyzer.

**ESTIMATION OF SERUM INSULIN**

**Methodology:**

Enzyme linked immunosorbent assay method using Insulin ELISA kit from DiaMetra.

Instrument:

Triturus analyzer – an open system automatic ELISA analyzer.

**ESTIMATION OF PLASMA GLUCOSE**

**Kit used:** Erba Mannheim XL Systems Packs

**Method:** Glucose oxidase – Peroxidase (GOD-POD) method – 1-point assay

**ESTIMATION OF SERUM TRIGLYCERIDES**

**Kit used:** Erba Mannheim XL Systems Packs

**Method:** Enzymatic method, end point

**ESTIMATION OF SERUM TOTAL CHOLESTEROL**

**Kit used:** Erba Mannheim XL Systems Packs

**Method:** Enzymatic method

**ESTIMATION OF SERUM HDL CHOLESTEROL**

**Kit used:** Erba Mannheim XL Systems Packs

**Method:** Immuno-inhibition method

**DETERMINATION OF LDL CHOLESTEROL**

LDL cholesterol was determined using the Freidwald's formula

LDL-c = Total cholesterol – (HDL-c + VLDL-c)

$$\text{VLDL-c} = \frac{\text{Triglycerides}}{5}$$

**DETERMINATION OF HOMA-IR**

$$\text{HOMA-IR} = \frac{\text{Serum insulin } (\mu\text{IU/mL}) \times \text{Plasma Glucose (mmol/L)}}{22.5}$$

**RESULTS & STATISTICAL ANALYSIS**

The mean and standard deviation for the various parameters evaluated are presented in Table 1

**TABLE No. 1 MEAN VALUE OF PARAMETERS AMONG THE VARIOUS GROUPS**

| | CONTROL | OVERALL TYPE 2 DM | TYPE 2 DM WITHOUT CAD | TYPE 2 DM WITH CAD |
|---|---|---|---|---|
| Age (yrs) | 49.92 ± 5.75 | 51.42 ± 3.48 | 50.94 ± 5.61 | 51.79 ± 3.44 |
| Male: female ratio | 28 : 22 | 60 : 50 | 26 : 22 | 34 : 28 |
| Duration of Diabetes (yrs) | ------ | 8.74 ± 2.89 | 8.33 ± 3.15 | 9.05 ± 2.98 |
| Body mass index kg/m2 | 24.26 ± 1.37 | 25.54 ± 2.16 | 24.26 ± 2.08 | 26.54 ± 2.35 |
| Fating Plasma Glucose mg/dL | 90.80 ± 5.71 | 153.16 ± 36.09 | 152.38 ± 44.92 | 153.77 ± 42.54 |
| Post-prandial Plasma Glucose mg/dL | 134.68 ± 6.11 | 218.29 ± 56.28 | 215.38 ± 69.11 | 220.55 ± 64.41 |
| Serum Triglycerides mg/dL | 134.66 ± 48.93 | 162.91 ± 54.40 | 150.94 ± 34.56 | 172.18 ± 53.80 |
| Serum Total Cholesterol mg/dL | 172.66 ± 21.25 | 203.46 ± 67.10 | 182.69 ± 51.82 | 219.55 ± 65.37 |
| Serum HDL-cholesterol mg/dL | 44.82 ± 6.07 | 39.87 ± 7.63 | 42.27 ± 6.36 | 38.02 ± 7.66 |
| Serum LDL-cholesterol mg/dL | 100.91 ± 24.02 | 131.01 ± 65.37 | 110.23 ± 49.42 | 147.10 ± 63.57 |
| Serum Insulin µIU/mL | 8.85 ± 1.20 | 8.80 ± 2.39 | 8.36 ± 2.90 | 9.15 ± 2.33 |
| HOMA -IR | 1.99 ± 0.34 | 3.20 ± 1.21 | 2.91 ± 0.68 | 3.43 ± 1.21 |

Descriptive statistical analysis was done by one way ANOVA F-test for p-value. The comparison of levels of each parameter among the various groups was analysed by Bonferroni t-test. Chi square test was done to analyse the male: female ratio in various groups. Karl Pearson's correlation coefficient was used to analyse the correlation between various parameters. 'P' value of 0.05 was considered to be significant.

Serum adiponectin and insulin resistance levels among the various groups studied are presented in Tables 2 and 3 respectively.

**TABLE No. 2 SERUM ADIPONECTIN LEVELS AMONG THE VARIOUS GROUPS**

| | Group | N | Mean | Std. Deviation | One way ANOVA F-test | Multiple comparison Bonferroni t-test |
|---|---|---|---|---|---|---|
| SERUM ADIPONECTIN µg/mL | 0 | 50 | 12.384 | 2.1287 | F = 78.5 P = 0.001 | 0 Vs 1,2 |
| | 1 | 48 | 7.088 | 2.7039 | | |
| | 2 | 62 | 6.442 | 2.9938 | | |
| | Total | 160 | 8.492 | 3.7408 | | |

**TABLE No. 3 INSULIN RESISTANCE AMONG THE VARIOUS GROUPS**

| | Group | N | Mean | Std. Deviation | One way ANOVA F-test | Multiple comparison Bonferroni t-test |
|---|---|---|---|---|---|---|
| HOMA-IR | 0 | 50 | 1.99 | 0.3406 | F = 39.41 P = 0.001 | 0 Vs 1,2 1 Vs 0,2 |
| | 1 | 48 | 2.908 | 0.6758 | | |
| | 2 | 62 | 3.434 | 1.2069 | | |
| | Total | 160 | 2.825 | 1.0468 | | |

**DISCUSSION**

One way ANOVA F-test was used analyse whether the three groups were age matched. The mean age of the control group (group 0), was 49.92 ± 5.749 years. For the group composed of Type 2 DM subjects without CAD (group 1) it was 50.94 ± 5.61 years and in the group composed of Type 2 DM with CAD it was 51.79 ± 3.44 years. All the three groups were found to be age matched. (p = 0.14)

Similarly chi-square test was performed to find out any difference in the male: female composition among the three groups. It was found out that the three groups were sex matched. (p = 0.98)

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The mean BMI in control group was 24.26 ± 1.37 kg/m2, in group 1 was 24.26 ± 2.08 kg/m2 and in group 2 it was 26.54 ± 2.35 kg/m2. The control group and group 1 were found to be similar as far as BMI was concerned. Group 2 subjects had a significantly higher BMI when compared to the other groups.

One way ANOVA F-test and multiple comparison Bonferroni t-test were used to analyse the serum adiponectin and insulin resistance levels among the three groups.

The mean serum adiponectin values of group 1 (7.088 ± 2.7 μg/mL) and group 2 (6.442 ± 2.99 μg/mL) were found to be significantly decreased (p = 0.001) when compared to the control group (12.384 ± 2.12 μg/mL).

Plasma adiponectin levels were decreased in obesity. This reduction may play a causal role in the development of insulin resistance. Obesity decreased the expression levels of adiponectin receptors, thereby reducing adiponectin sensitivity, which finally leads to insulin resistance, the so-called "vicious cycle" (10). Both genetic and environmental factors play a role in hypoadiponectinemia in diabetes mellitus (14). Plasma adiponectin levels have been shown to be decreased in cardiovascular diseases (11, 12). The mean adiponectin level of the diabetes mellitus subjects (group 1 and 2) was found to be significantly decreased when compared to the control group. It was also found that group 2 subjects who had higher BMI had significantly lower serum adiponectin when compared to the other groups.

Insulin resistance as measured by HOMA-IR was found to be significantly increased in group 1 and group 2 when compared with the control group. When group 1 and group 2 were compared, group 2 had significantly higher insulin resistance (p = 0.001).

Karl Pearson correlation coefficient was used to analyse the correlation between serum adiponectin and other parameters.

Serum adiponectin in the control group had a significantly positive correlation with serum HDL cholesterol and significant negative correlation with BMI, fasting plasma glucose, serum total cholesterol, LDL cholesterol and insulin resistance.

Subjects with higher serum adiponectin levels had more favourable parameters namely higher HDL cholesterol and lower serum total cholesterol, LDL cholesterol and insulin resistance.

Serum adiponectin in the group 1 had a significantly positive correlation with HDL cholesterol and a significantly negative correlation with insulin level and insulin resistance.

Serum adiponectin in the group 2 had a significantly positive correlation with HDL cholesterol and a significantly negative correlation with BMI, post prandial plasma glucose, serum triglycerides, total cholesterol, LDL cholesterol, insulin level and insulin resistance.

Serum adiponectin level is negatively correlated with BMI, serum insulin levels and insulin resistance in the entire study group and was significantly reduced in group 1 and group 2. The secretion of adiponectin by adipocytes appears to be hormone regulated. Adiponectin expression declines following stimulation with insulin, TNF- , endothelin-1, and glucocorticoids, whereas transcription appears to increase with IGF-I treatment (13).

**CONCLUSION**

From the analysis of the data, the following conclusions were made:

1. Serum adiponectin level was decreased in Type 2 diabetes mellitus subjects when compared with controls. In Type 2 diabetes mellitus subjects with coronary artery disease, the serum adiponectin level was decreased when compared with those without coronary artery disease.

2. Serum adiponectin was negatively correlated with serum insulin level and insulin resistance in all the groups studied.

3. Serum adiponectin was negatively correlated with the body mass index in the control and Type 2 diabetes mellitus.

4. Serum adiponectin had a moderate, negative correlation with fasting plasma glucose, serum total cholesterol and LDL cholesterol. There was a moderate, positive correlation between serum adiponectin and HDL cholesterol in the controls and a substantial, positive correlation in Type 2 diabetes with CAD.

5. Subjects with higher serum adiponectin levels had more favourable parameters namely higher HDL cholesterol and lower levels of serum total cholesterol, LDL cholesterol, and insulin resistance.

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