Study of Serum Adiponectin, Insulin and Insulin Resistance in Offsprings of Diabetic Parents

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ABSTRACT
Adiponectin is an adipocytokine that is exclusively expressed and secreted from adipose tissue. Its role as a protective adipokine is suggested by low levels in states of insulin resistance (IR), cardiovascular disease (CVD) and type 2 diabetes (T2DM). In present study we compared serum adiponectin, insulin and (IR) in offsprings’ of diabetic and non-diabetic parents and showed if any significant difference is there between both the groups. Analytical cross-sectional study was carried out. Subjects were randomly selected based on inclusion and exclusion criteria aged between 18-30 years and grouped in three groups: Group 1: 60 off-spring of non diabetic parents; Group 2: 38 off-spring of single diabetic parents; Group 3: 22 off-spring of both diabetic parents. In all the groups fasting plasma glucose, serum insulin and serum adiponectin were estimated. Statistical analysis was carried out to compare values with group 3. Mean values of serum adiponectin were Group 1 (10.25 µg/ml ± 1.68), group 2 (9.25 µg/ml ± 1.81) and group 3 (10.86 µg/ml ± 1.23), Fasting blood sugar mean value in group 1: (86.84 mg/dl ± 6.25), group 2: (94.95 mg/dl ± 6.58), group 3: (85.23 mg/dl ± 5.93), Insulin mean values in group 1 (10.81 µU/mL ± 1.73), group 2: (12.69 µU/mL ± 1.55), group 3 (8.69 µU/mL ± 2.55), Insulin resistance group 1 (2.4±0.48), group 2 (2.55±0.55), group 3: (1.8±0.55). The values of all the three parameters were significantly different between three groups (p< 0.01). Serum level of adiponectin, insulin and insulin resistance were significantly different in offsprings’ of diabetic parents. Serum Adiponectin might play a key role in developing diabetes in near future.

KEY WORDS: adiponectin, cardiovascular disease (CVD), insulin resistance (IR).

INTRODUCTION:
Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both and is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Type 2 diabetes is increasing all over the world due to population growth, aging, urbanization and an increase of obesity and physical inactivity.

In India, more than 62 million people are diagnosed as diabetic which will rise to 87.0 million by 2030. It has been reported that diabetes is disproportionately high in young to middle aged adults which could have long lasting adverse effects on a nation’s health and economy. First degree relatives of patients with type 2 diabetes frequently show abnormal glucose tolerance and have a 30% - 40% risk of developing type 2 diabetes themselves.

Adiponectin, is a novel peptide expressed specifically and abundantly in adipose tissue. Adiponectin is a 244 a.a. protein that regulates the metabolism of lipids and glucose. Adiponectin decreases insulin resistance and body weight by increasing lipid oxidation in muscle and other organs such as the pancreas and liver. Plasma Adiponectin concentrations are also reduced in individuals with obesity, diabetes mellitus, or coronary heart disease.

It seems to be the most interesting and promising biologically active molecule released from fat cells which has profound protective actions in the pathogenesis of diabetes mellitus and cardiovascular disease.
There are few studies which have shown hypo-adiponectinemia is associated with family history of diabetes but not all. Purpose of this study was to investigate any significant difference in the level of total serum adiponectin and their association with fasting insulin, glucose and insulin resistance in offspring of diabetics.

MATERIALS AND METHODS:
Analytical cross sectional study was carried out after the approval by Institutional ethics committee of People's College of Medical Sciences & Research Center, Bhopal. Written consent was taken from all participants. Total 120 subjects in which 60 normal healthy offspring of diabetic parents and 60 normal healthy offspring of non-diabetic parents between age group of 18 to 30 years were enrolled after applying all inclusion and exclusion criteria. All socio-demographic data of the participants were entered in a self-designed proforma.

Inclusion criteria:
1. Normal healthy off-springs of diabetic parents in age group 18 to 30 yrs.
2. Normal healthy off-springs of non-diabetic parents in age group 18 to 30yrs.

Exclusion criteria:
1. Subjects not fulfilling the age criteria mentioned in inclusion criteria.
2. Any acute illnesses, such as infection, surgery, and hospital admission (as obtained from history).
3. Subjects with fasting blood sugar level >110 mg/d

Participants were divided into three groups:
Group 1: control group consists (n=60) both male and female of age group between 18 to 30 years, whose parents are non-diabetic.
Group 2: (n=38) both male and female of age group between 18 to 30 years, with one of their parents having history of type 2 diabetes.
Group 3: (n=22) both male and female of age group between 18 to 30 years with both parents having history of diabetes.

Weight and height were measured. BMI was evaluated as weight divided by height squared (kg/m²). Waist circumference was measured at a level midway between the lowest rib margin and the iliac crest. Hip circumference was measured at the widest point over the buttocks. WHR (waist hip ratio) were calculated as waist circumference divided by hip circumference.

After overnight fasting (8-12 hrs.) blood sample for plasma glucose was collected in fluoride vial for blood sugar and that of insulin and adiponectin were collected in plain vial between 8 am-10 am. Sample was centrifuged; serum was separated and immediately stored in deep freezer at -20°C until further analysis.

Serum Glucose was measured by commercial kits for Bio system A25 fully auto analyzer. The plasma insulin and serum Adiponectin was analyzed by using ELISA method. Insulin resistance was estimated using HOMA-IR based on fasting plasma glucose (FPG) and fasting plasma insulin (FPI) concentrations. Which is defined as follows: FPI (μU/mL) x (FPG [mg/dl] x 0.055/22.5).

STATISTICAL ANALYSIS:
In the current study we calculated mean value of serum adiponectin, insulin and insulin resistance in 3 groups. Comparison between the groups was done by applying ANOVA and Post hoc analysis. Multiple regression was performed to see the correlation between adiponectin and other biomarkers keeping the adiponectin as dependent variable. The collected data was analyzed by SPSS 20 version with assistance from a qualified statistician. p value<0.05 were considered significant difference.

RESULTS:
In our study we found significant difference between mean values of Fasting blood Glucose in 3 groups (Group1- 86.84 mg/dl ± 6.2, Group 2-94.95 mg/dl ± 6.5, Group3- 85.23 mg/dl ± 5.9; p = 0.000**). The mean value of Fasting Blood Sugar was higher in offspring of both diabetic parents than offspring of single diabetic parent and the difference was highly significant (p=0.000**). The mean value of Fasting Blood Sugar was higher in offspring of both diabetic parents than offspring of non-diabetic parent and the difference was also statistical significant (p=0.000**). There was no statistical significant difference in mean value of Fasting Blood Sugar between offspring of single diabetic parent and offspring of non-diabetic parent (p= 0.421) (Table 1 andTable 2).

There was a significant difference between mean values of Serum Insulin in all groups (Group1- 10.81 μU/mL ± 1.7, Group2- 12.7 μU/mL ± 1.5, Group3-8.69 μU/mL ± 2.5; p=0.000**). The mean value of Serum Insulin was Higher in offspring of both diabetic parents than offspring of non-diabetic parent and the difference was statistical significant (p=0.000**). The mean value of Serum Insulin was
higher in offspring of both diabetic parents than (Group1- 1.8 ± 0.5, Group2- 2.4 ± 0.4, Group3- 2.5 ± 0.5; p= 0.000**). The mean value of Insulin Resistance was higher in offspring of both diabetic parents than in offspring of non-diabetic parent and the difference was statistical significant (p=0.000**). The mean value of Insulin Resistance in offspring of single diabetic parent was higher than in offspring of non-diabetic parent which was found to be highly significant (p=0.000**). The mean value of Insulin Resistance was higher in offspring of both diabetic parents than offspring of single diabetic parent but the difference was not statistical significant (p=0.566) (Table 1 and Table 2).

For these 3 groups the mean values of serum adiponectin (µg/ml) were compared (Group1- 10.25 µg/ml ± 1.68, Group2 - 9.25 µg/ml ± 1.81, Group3- 10.86 µg/ml ± 1.23) and this difference was highly significant. The mean value of serum adiponectin was lower in offspring of both diabetic parents than offspring of single diabetic parent and offspring of non-diabetic parents. The difference between group 3 and group 1 was statistical significant (p=0.000**). However, the difference between group 3 and group 2 was also statistical significant (p=0.039*). The mean value of Serum Adiponectin was lower in offspring of single diabetic parent than offspring of non-diabetic parents but the difference was found to be statistically non significant (p=0.124) (Table 1 and Table 2).

Table 3 shows correlation of adiponectin with other parameters using multiple regression analysis. The value of serum adiponectin was negatively correlated with BMI, Waist hip ratio, FBS, Serum Insulin and insulin resistance. Negative correlation means increase in value of Serum adiponectin was associated with decrease in values of BMI, Waist hip ratio, FBS and Serum Insulin and insulin resistance. The correlation between serum adiponectin and BMI, FBS, and serum insulin was statistical significant (p<0.05) but that with W/H ratio and insulin resistance was statistically insignificant.

**DISCUSSION:**

Type 2 diabetes is a metabolic syndrome which is relatively common in most countries including India which is now being referred to as “Diabetes Capital of the world” and risk of becoming diabetic for individual increases by two to four times if he has positive family history. Adiponectin is a new marker which is found associated with type 2 diabetes however it is not clear whether it is associated with family history of diabetes.

In the current study we measured fasting blood glucose, serum insulin, BMI, Waist Hip ratio, serum Adiponectin and through mathematical model for insulin resistance HOMA-IR in the first degree relatives of diabetes patient and tried to assess the association of adiponectin in future onset of diabetes.

Although in our study the fasting blood glucose level in all the three groups was within normal range but we found highly significant difference observed in mean value of blood glucose between offspring of both diabetic parents and offspring of non diabetic parent (p=0.000**). There was also highly significant difference between offspring of both diabetic parents and offspring of single diabetic parent (p=0.000). In support with our study Shahid et al (15) also found significantly higher level of fasting blood glucose in offspring of both diabetic parents compared to offspring of single and offspring of non-diabetic parents.

Table 3: Correlation between Serum Adiponectin and other biomarkers using Multiple Regression Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>R</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>-0.368</td>
<td>-0.761</td>
<td>-13.608</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>W/H ratio</td>
<td>-2.949</td>
<td>-0.097</td>
<td>-1.780</td>
<td>0.078</td>
</tr>
<tr>
<td>FBS</td>
<td>-0.026</td>
<td>-0.116</td>
<td>-2.175</td>
<td>0.032*</td>
</tr>
<tr>
<td>Insulin</td>
<td>-0.134</td>
<td>-0.221</td>
<td>-3.278</td>
<td>0.001*</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>-0.088</td>
<td>-0.034</td>
<td>-0.513</td>
<td>0.609</td>
</tr>
</tbody>
</table>

[* p value <0.05 (significant), ** p value <0.01 (highly significant)*]
offspring of single diabetic parent which was highly significant statistically (p=0.004**). The mean value of Serum Insulin was higher in offspring of single diabetic parent than offspring of non-diabetic parent and the difference was statistical significant (p=0.000**). A highly significant difference between mean values of insulin resistance was found in all groups.

Table 1: Mean value ±SD serum adiponectin and other biomarkers in study groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (n=38)</th>
<th>Group 2 (n=22)</th>
<th>Group 3 (n=60)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood Glucose (mg/dl)</td>
<td>85.23 ± 5.9</td>
<td>86.84 ± 6.2</td>
<td>94.95 ± 6.5</td>
<td>0.000**</td>
</tr>
<tr>
<td>Fasting serum Insulin (µU/mL)</td>
<td>8.69 ± 2.5</td>
<td>10.81 ± 1.7</td>
<td>12.7 ± 1.5</td>
<td>0.000**</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.8 ± 0.5</td>
<td>2.4 ± 0.4</td>
<td>2.5 ± 0.5</td>
<td>0.000**</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>10.86 ± 1.2</td>
<td>10.25 ± 1.7</td>
<td>9.25 ± 1.8</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

(* p value <0.05(significant), ** p value <0.01 (highly significant)) HOMA-IR- Homeostasis model assessment-insulin resistance

Table 2: Comparison between 3 groups using Post hoc analysis of ANOVA test

<table>
<thead>
<tr>
<th>Variable</th>
<th>A</th>
<th>B</th>
<th>Mean Difference (A-B)</th>
<th>Std. Error</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Adiponectin (µg/ml)</td>
<td>Group 1</td>
<td>Group 2</td>
<td>0.61333</td>
<td>0.31137</td>
<td>0.124</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td></td>
<td>1.60879*</td>
<td>0.37433</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Group 2</td>
<td>Group 1</td>
<td>-0.61333</td>
<td>0.31137</td>
<td>0.124</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td></td>
<td>0.99545*</td>
<td>0.40235</td>
<td>0.039*</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td>Group 1</td>
<td>-1.60879*</td>
<td>0.37433</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Group 2</td>
<td></td>
<td>-0.99545*</td>
<td>0.40235</td>
<td>0.039*</td>
</tr>
<tr>
<td>Serum Insulin (µU/mL)</td>
<td>Group 1</td>
<td>Group 2</td>
<td>-2.12175*</td>
<td>0.44782</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td></td>
<td>-3.99424*</td>
<td>0.53837</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Group 2</td>
<td>Group 1</td>
<td>2.12175*</td>
<td>0.44782</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td></td>
<td>-1.87249*</td>
<td>0.57867</td>
<td>0.004**</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td>Group 1</td>
<td>3.99424*</td>
<td>0.53837</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Group 2</td>
<td></td>
<td>1.87249*</td>
<td>0.57867</td>
<td>0.004**</td>
</tr>
<tr>
<td>Insulin Resistance</td>
<td>Group 1</td>
<td>Group 2</td>
<td>-0.6060*</td>
<td>0.1105</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td></td>
<td>-0.7515*</td>
<td>0.1329</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Group 2</td>
<td>Group 1</td>
<td>0.6060*</td>
<td>0.1105</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td></td>
<td>-0.1456</td>
<td>0.1428</td>
<td>0.566</td>
</tr>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
<td>0.7515*</td>
<td>0.1329</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td></td>
<td>0.1456</td>
<td>0.1428</td>
<td>0.566</td>
</tr>
</tbody>
</table>
Although we did not found any case of diabetes in the offspring, we found highly significant difference in mean value of blood glucose between offspring of both diabetic parents and offspring of non-diabetic parent (p=0.000**). As reported by Lihn et al [16] at baseline the subjects of his study, and in which they describe that those patients, who progressed to diabetes, increased their levels at a rate of 2.27mg/dl per year. So probability of developing diabetes at later stage in these group will be more.

In our study we found significantly higher level of Serum Insulin in offspring of both diabetic parents than offspring of non-diabetic parent (p=0.000**) and also Serum Insulin was significantly higher in offspring of both diabetic parents than offspring of single diabetic parent (p=0.004**). The mean value of Serum Insulin was also significantly higher in offspring of single diabetic parents than offspring of non-diabetic parent (p=0.000**).

Higher insulin levels in the offspring of both diabetic parents were consistent with studies referred by costa et al [17] basal insulin levels were higher in normo-glycemic first degree relatives (2.4 ± 0.4 μU/ml) than corresponding values in control (2.1 ± 0.6 μU/ml). Adeela Shahid et al [15] also found significantly higher level of insulin concentration in offspring’s of both diabetic parents compared to non-diabetic parents.

It is known that type 2 diabetes frequently shows a resistance to the effect of insulin. [18] Although the etiology is not clear, there is no autoimmune destruction of the beta cell in type 2 diabetes. Researches carried out on family members with diabetes and twins with diabetes confirmed that hereditary factors play a role in type 2 diabetes.

We estimated Insulin resistance by HOMA-IR which has been shown to correlate well with clamp methods, we found that IR was significantly high in offspring’s of both diabetic parents compared to offspring of non-diabetic parents (p=0.000**). The mean value of IR was also significantly higher in offspring of single diabetic parent compared to the value in offspring of non-diabetic parents (0.000**).

Insulin resistance and defective insulin secretion are considered to be the most important events in the development of NIDDM, and insulin resistance would represent a genetic determinant of NIDDM study population. [18-21]

We found that the mean value of serum Adiponectin was significantly lower in offspring of both diabetic parents than offspring single diabetic parent (0.039*) and offspring of non-diabetic parents (0.000**). In comparison with our study, Pellme F et al [22] and Sull et al [23] also found that family history of diabetes is associated with hypo-adiponectinemia.

Lindsay et al [24] recently showed in a case-control study with Pima Indians that higher plasma levels of adiponectin protected against later development of type 2 diabetes.

Lihn et al [16] found that adiponectin expression in adipose tissue is reduced in first degree relatives. He found substantially reduced levels of adiponectin mRNA in subcutaneous adipose tissue from first degree relatives compared with control subjects.

To find correlation of serum adiponectin with other parameters we applied multiple regression analysis. We found that serum adiponectin was inversely correlated with BMI and was significant but correlation with WHR was not significant. Our findings are consistent with Indian studies reported by researchers Vikram et al [25] found that adiponectin levels correlate (inversely) strongly with anthropometric parameters in Asian-Indians though unlike their study, our study does not show a strong correlation with WHR.

In our study serum adiponectin concentrations also inversely correlated with fasting blood glucose and insulin which is consistent with other studies referred by Bacha et al [26], and recently Bose et al [27] who also showed negative correlation of serum adiponectin with insulin. Plasma adiponectin levels were inversely correlated with fasting glucose and insulin study done by Hotta k et al [28]. Weyer C et al.

Comuzzie AG et al [29] in his study that Adiponectin levels reported genetic heritability of 46%. Genetic polymorphisms in the adiponectin gene have been identified [30, 31] and shown to be associated with obesity and insulin resistance. [32] Yet, the mechanism by which adiponectin influences insulin sensitivity in humans is unclear.

The main limitation of this study is that it is a cross-sectional study. In our study we had taken the sample from medical and dental students. Thus our study shows sample bias and findings do not represent the community. As the rates of the variables under study are fairly high in the community, our sample size was small. To generalize the findings a larger population study needs to be carried out.

CONCLUSION:

These observations suggest that increased circulating adiponectin values may serve as a useful marker of predisposition to type 2 diabetes among
offspring of diabetic parents. We suggest further studies an important marker in larger samples at various centers at national and international level so that exact role of serum adiponectin as a screening tool for type 2 diabetes in vulnerable population could be established.

REFERENCES:

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