

Biochemistry

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BIOMARKER FOR INSULIN RESISTANT IN TYPE 2 DIABETES AMONG SOUTH INDIANS



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ABSTRACT:

OBJECTIVE: In Type 2 diabetes, Insulin resistant leads microvascular and macrovascular complication. Early finding prevents from this complication and it is a challenging task. A decade research shows adiponectin may be a marker for insulin resistant. Increasing the level of adiponectin prevent the progression of insulin resistant(IR). In this research we are striving adiponectin as a biomarker for IR was assessed through receiver operating characteristic curve(ROC) also associating the correlation with lipid parameter in the South Indian population.

MATERIALS AND METHODS: 30 Type 2 Diabetic patients, 20 Controls were studied. Fasting serum sample were used to measure Adiponectin, Insulin. Fasting plasma sample were used to measure Glucose. Anthropometric data including height, weight, BMI and lipid parameters(HDL, LDL) were measured using a standard technique.

RESULT: The receiver operating characteristic curve (ROC) shows moderate discrimination for Adiponectin as a biomarker. The Adiponectin assay has Sensitivity(40%), Specificity(70%), Positive likelihood ratio(1.33), Negative likelihood ratio(0.86), Disease prevalence(60%), Positive predictive value(66.67%), Negative predictive value(43.75%), Accuracy(52%). The reduction of mean adiponectin value observed in study group compared to control group. Also, the correlation of Fasting blood sugar(FBS) and Adiponectin in control is negative correlation, whereas in study group its positive correlation but both the subjects its highly significant. For lipid parameters, correlation of HDL and LDL with adiponectin in control and study group is positive correlation also its highly significant. For the prediction of insulin resistant biomarker, Adiponectin yielded on ROC-AUC of 0.46, which is a moderate agreement.

CONCLUSION: Adiponectin might be a prediction for biomarker in Type 2 diabetes associated insulin resistant among South Indians if test has been done with the high specificity and sensitivity of the ELISA kit to arrive good discrimination.

INTRODUCTION

β cell dysfunction is one of the major cause for insulin resistance in type 2 diabetes, also in some cases longstanding stress causes the elevation of cortisol, higher level of this hormone damages the β cell by the way decreases insulin production and increases the insulin resistant (1). Prolonged exposure of high level of fatty acids accumulates in the muscle and liver, causes the inhibition of glucose to enter in both tissues, in response more amount of insulin produced (Hyperinsulinemia) to unlock the cell for utilisation of

glucose. Frequent and longstanding of this type of mechanism end in apoptosis of islet cell also no more overproducing insulin to meet the requirement of muscle and liver which is also causative for insulin resistant (2,3). More researches on central obesity reports, visceral lipid deposits are increased and the abdominal subcutaneous adipose tissue depot is decreased, in type 2 associated insulin resistance in Asian Indians. Recent researches emphasised the involvement of adiponectin, one of the adipokines released from adipose tissue that regulates the metabolism of lipids and glucose also increases the insulin sensitivity (4,5). In this research we are striving to find out the adiponectin as a Biomarker for insulin resistant in type 2 diabetes among South Indians, also finding the correlation between the lipid analytes. Early finding of insulin resistant through a biomarker will help to prevent from microvascular and macrovascular complications (6).

MATERIALS AND METHODS

The study has been conducted at D.A.E. Township, Kalpakkam, Kancheepuram District as a Case-control study. Age range from 40 to 60 years including both genders categorised Type 2 diabetes (Study group) and Non-diabetes (Control group). Written informed consent was obtained from each participant before commencement of the study.

The sample included with 20 normal healthy individuals, and the 30 study group of known Type 2 diabetes. Before blood collection Individuals Age, Sex, Height, Weight noted. BMI was calculated using Height and Weight measurement (7). 10 – 12 hours overnight fasting instructed for both the subjects. After overnight fasting Venous blood drawn from individuals and 2 ml transferred in sodium fluoride/ Na_2EDTA anticoagulated tubes for fasting blood glucose estimation, remaining 6 ml blood was transferred in plain tubes(without anticoagulant) for estimation of serum fasting insulin, serum adiponectin, HDL and LDL. All the samples were centrifuged at 2000 rpm for 10 minutes, and the fasting blood glucose estimation done by glucose oxidase/ peroxidase (GOD/POD) method, Serum insulin measured by chemiluminescence method, Serum Triglyceride measured by enzymatic method i.e. GPO - PAP (Glycerol phosphate oxidase – peroxidase – amidopyrine method), Serum HDL measured by Cholesterol Esterase, Peroxidase end point method (10, 11). serum adiponectin measured using DBC (Diagnostics Biochem Canada) ELISA Kit (8, 9).

RESULTS

A significant increase of insulin, and highly significant increase in fasting blood glucose in diabetic (study group) compared with control group confirms the selected study group are insulin resistant. The low mean concentration value of adiponectin, HDL in study group compared with control group observed. In contrast, the mean concentration of LDL decreased in study group.

TABLE – 1 COMPARISON OF PARAMETERS IN THE CONTROL AND STUDY GROUPS

PARAMETER	CONTROL	STUDY	P Value
Age	46.55 ± 5.8	47.9 ± 5.8	0.428
BMI (Kg/m ²)	24.75 ± 3.6	25.03 ± 3.5	0.786
ADIPONECTIN (µg/mL)	4.12 ± 2.2	3.95 ± 2.2	0.800
FBS (Mg/dl)	103.35 ± 12.5	192.06 ± 72.6	<0.0001 **
INSULIN (µIU/MI)	8.72 ± 3.2	13.86 ± 9.6	0.026 *
HDL (Mg/dl)	41.3 ± 13.6	35.8 ± 6.9	0.065
LDL (Mg/dl)	110.9 ± 38.02	94.3 ± 28.03	0.082

* significant (P < 0.05) *Highly significant

TABLE – 2 CORRELATION OF FASTING BLOOD SUGAR WITH ADIPONECTIN, FASTING INSULIN, BMI CONTROL AND STUDY GROUP

PARAMETER	CONTROL	r	STUDY	r	P Value
Adiponectin	4.12 ± 2.2	- 0.401	3.95 ± 2.2	0.058	0.0001*
Fasting Insulin	8.72 ± 3.2	0.069	13.8 ± 9.6	- 0.281	0.0001*
BMI	24.75 ± 3.6	0.135	25.03 ± 3.5	- 0.207	0.0001*

*Highly significant r – correlation coefficient

TABLE – 3 CORRELATION OF HDL WITH ADIPONECTIN IN CONTROL AND STUDY GROUP

PARAMETER	CONTROL	r	STUDY	r	P Value
ADIPONECTIN	4.12 ± 2.2	0.659	3.95 ± 2.2	0.328	0.0001*

*Highly significant r – correlation coefficient

TABLE – 4 CORRELATION OF LDL WITH ADIPONECTIN IN CONTROL AND STUDY GROUP

PARAMETER	CONTROL	r	STUDY	r	P value
ADIPONECTIN	4.12 ± 2.2	0.296	3.95 ± 2.2	0.082	0.0001*

*Highly significant r – correlation coefficient

In respect of correlation studies, highly significant negative correlation of adiponectin (r = - 0.401, p<0.0001) with fasting blood glucose, highly positive correlation of fasting insulin (r = 0.069, p<0.0001) and BMI (r = 0.135, p<0.0001) with fasting blood glucose in control group were observed.

In study group highly significant positive correlation of adiponectin (r = 0.058, p<0.0001) with fasting blood glucose, highly negative correlation of fasting Insulin (r = - 0.281, p<0.0001) and BMI (r = - 0.207, p<0.0001) with fasting blood glucose were noticed.

One of the Type 2 associated complication is atherosclerosis(16). Adiponectin shows highly significant positive correlation with HDL, LDL (atherosclerosis marker) (r = 0.659, r = 0.296, p<0.0001) in control group and in study group also adiponectin shows highly significant positive correlation (r = 0.328, r = 0.082, p<0.0001).

TWO-WAY FREQUENCY TABLE

To evaluate any diagnostic test or striving to predict as a biomarker, its best summarised by two-way frequency table. In this research for biomarker in insulin resistant type 2 diabetes, two - way table drawn for adiponectin as a diagnostic biomarker and gold standard as Fasting blood glucose. (14).

TABLE – 5 TWO – WAY FREQUENCY TABLE

Diagnostic Test (Adiponectin)	Gold Standard (FBS)		Total
	Positive	Negative	
Positive	12 (a)	06 (c)	18
Negative	18 (b)	14 (d)	32
Total	30 (a+b)	20 (c+d)	50

From the above table, calculated parameters relevant to predicted biomarker(Adiponectin) as follows:

Statistic	Formula	Value	95% Confidence Interval (CI)
Sensitivity	$\frac{a}{a+b}$	40.00%	22.66% to 59.40%
Specificity	$\frac{d}{c+d}$	70.00 %	45.72% to 88.11%
Positive Likelihood Ratio	$\frac{\text{Sensitivity}}{1 - \text{Specificity}}$	1.33	0.60 to 2.97
Negative Likelihood Ratio	$\frac{1 - \text{Sensitivity}}{\text{Specificity}}$	0.86	0.57 to 1.29
Disease prevalence	$\frac{a+b}{a+b+c+d}$	60.00%	45.18% to 73.59%
Positive Predictive Value	$\frac{a}{a+c}$	66.67%	47.33% to 81.66%
Negative Predictive Value	$\frac{d}{b+d}$	43.75 %	34.06% to 53.95%
Accuracy	$\frac{a+d}{a+b+c+d}$	52.00%	37.42% to 66.34%

Definitions

Sensitivity: probability that a test result will be positive when the disease is present (true positive rate). **Specificity:** probability that a test result will be negative when the disease is not present (true negative rate). **Positive likelihood ratio:** ratio between the probability of a positive test result given the *presence* of the disease and the probability of a positive test result given the *absence* of the disease, i.e. = True positive rate / False positive rate = Sensitivity / (1- Specificity) **Negative likelihood ratio:** ratio between the probability of a negative test result given the *presence* of the disease and the probability of a negative test result given the *absence* of the disease, i.e. = False negative rate / True negative rate = (1-Sensitivity) / Specificity **Positive predictive value:** probability that the disease is present when the test is positive. **Negative predictive value:** probability that the disease is not present when the test is negative. **Accuracy:** overall probability that a patient will be correctly classified.

ROC curve

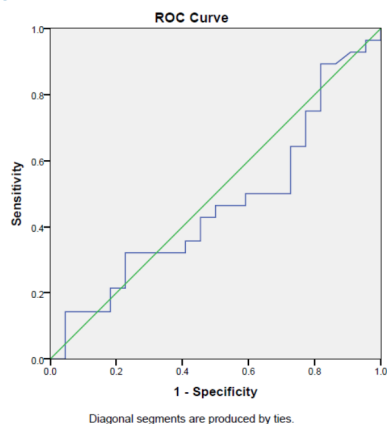
ROC curves are particularly useful for comparing different diagnostic tests and when more than one test is available they can be compared by plotting both on the same plot(Fig.1). A test for which the plot is consistently nearer the left-hand side and the top is to be preferred. In addition, the area under the curve (AUC) for each plot can be calculated. Here, we plotted as sensitivity versus one minus specificity for different cut-off values of the diagnostic test. The area under the curve we arrived is 0.465, which is a moderate agreement (12,13).

DISCUSSION

Decade research shows that Asian Indians have more insulin resistance (IR) than other ethnicity. Higher level of BMI (> 25), fasting blood sugar, fasting insulin are some of the causes for IR, but in South Indians insulin resistant observed due to central obesity (15). Adiponectin is a protein encoded by ADIPOQ gene, secreted from adipose tissue, involves in glucose and lipid level regulation. In the present study, the adiponectin in diabetic patients had a lower mean value than control group, and the study group adiponectin positively associated with HDL, both of these observations same like Leena chand, 2016 (20). Adiponectin level can be linked to whole-body insulin sensitivity, and hypoadiponectinemia can cause endothelial dysfunction by decreasing insulin sensitivity, also it may be directly linked to early atherosclerotic vascular damage and a subsequent endothelial dysfunction (17). Comparing study group fasting blood sugar mean value with control group and comparing study group fasting insulin mean value with control group shows higher and statistically significant. These observations seen same as Julian Esparza-Romero *et.al.*, 2010 (21). There is a negative correlation between fasting blood sugar and adiponectin in control group and positive correlation between fasting blood sugar and adiponectin in study group, both of them are statistically highly significant, the above observation confirms the insulin resistant also the adiponectin value will be low in insulin resistant cases.

In ROC analysis (Fig.1), the area under the curve we arrived is 0.465 according to the Sensitivity (40%), Specificity (70%) of the ELISA kit used. The factor which has been affecting the good agreement is sample size and selection of control group. The equal number of case – control sample will give the good discrimination with $AUC > 0.5$. Regarding control group, the selection of the age group was between 40 to 60 years, it's same as age in study group. In this age group, the presence of IGT (Pre-diabetic) stage samples not ruled out that's why we are not arriving significance while comparing adiponectin values with case-control samples though the control samples has higher mean value than study group (18, 19).

Fig. 1 Receiver operating characteristic curve (ROC) of the Adiponectin ELISA for the detection of Insulin resistant in Type 2 diabetes



CONCLUSION:

Serum Adiponectin level decreases with insulin resistance, also decreases in type 2 associated complication (Low level HDL, and High-level LDL). These observations were well correlated and adiponectin can be used as a biomarker in Type 2 associated insulin resistant and its complication with the high sensitivity and high specificity kit.

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