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Original Research Article

Association of adiponectin & leptin with insulin resistance in type-2 diabetes

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ABSTRACT

Introduction: Indian phenotype having greater abdominal adiposity, higher waist circumference (WC) & lower body mass index (BMI), thereby making the Indians more prone to diabetes and its complications. Type-2 diabetes mellitus (T2DM) worldwide known for its morbidity and mortality. Recent studies have demonstrated that the abnormal levels of adipocytokines (Adiponectin, leptin) may contribute to Insulin resistance (IR) and T2DM.

Aim: The present study aimed to analyze circulatory levels of Adiponectin & Leptin with Insulin Resistance in study subjects compared with control subject & Type-2 diabetes mellitus (T2DM).

Materials and Methods: A total 120 T2DM patients and 120 healthy subjects aged between 30 and 60 years who were Non-diabetic, Non-hypertensive and having no family history of hypertension visiting to Medicine OPD were included in study. Parameters such as age, sex, anthropometric measures and biochemical indicators like fasting and postprandial blood sugar, lipid profile, adiponectin, leptin, fasting insulin, HOMA-IR & systolic and diastolic blood pressure were determined & compared with control subjects.

Result: Adiponectin levels were reported significantly lower in T2DM group whereas Leptin levels were significantly higher in T2DM group compared to non-diabetics group ($P \leq 0.001$) in both genders.

Conclusion: Adiponectin & Leptin levels are used as an important clinical biomarkers for T2DM and obesity. IR, Adiponectin and Leptin can be used as cut off to predict the occurrence of T2DM.

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1. Introduction

Insulin resistance is a pathological state in which insulin action is impaired in target tissues including liver, skeletal muscle, and adipose tissue, thus any defect in the insulin signaling cascade can cause insulin resistance. Insulin resistance is a central abnormality of the metabolic syndrome, or syndrome X originally hypothesized by Reaven¹ to describe a constellation of metabolic abnormalities, including hyperglycemia, hyperinsulinemia, hypertension, dyslipidemia with increased triglycerides, and decreased HDL-C levels.

Diabetes mellitus is a metabolic disorder affecting millions of people and is leading cause of morbidity and mortality worldwide. T2DM is most common form of disease occurring in Study population.² India has earned distinction being a diabetes capital of the world.³ The International Diabetes Federation (IDF) estimates the number of diabetics in India to be 72.9 million in 2017 and this number is expected to increase to 134.3 million by the year 2045.⁴ Insulin Resistance (IR) is the main characteristic of T2DM and promotes multiple organs failure along with resistance of insulin in skeletal muscle, liver, adipose tissue. Onset of hyperglycemia and diabetes is often lead by several years of IR & obesity is an important phenomenon that provides a main link between T2DM and

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accumulation of fat.⁵

Adipocytokines are cytokines secreted by adipose tissue. Among other, they include adiponectin and leptin.⁶ Adiponectin generally modulates glucose regulation and fatty acid catabolism.⁷ Despite being generated in adipose tissue, adiponectin levels are reduced in obesity and the circulating levels of the adiponectin are correlated inversely with the body fat percentage in adults showing significant increase after weight reduction.⁸ The onset of overt diabetes takes place when β -cells of the pancreas can no longer effort excess insulin secretion in compensation of IR⁹ but exact mechanism which can lead to insulin resistance is still unknown. An association of Adiponectin and Leptin on human pathophysiology elicits considerable interest in view of their potential role as a remedial measure for obesity and other insulin resistant states. An IR is a pathological state in which insulin action is impaired in target tissues including skeletal muscle, liver and adipose tissue. Any defects in the insulin signaling cascade can cause IR. Adiponectin & leptin are the abundant adipocytokines generated by adipocytes as documented in the study done by Rajmangal choudhary 2019.¹⁰ Adipocytokines has an important role in various pathological conditions like deranged energy homeostasis, abnormal leukocyte migration and polycystic ovary. Adequate evidences are still lacking regarding the effects of the above molecules in the pathogenesis of IR.

The Leptin biochemically a 16 kDa protein product of Ob gene is an adipokine discovered in 1994¹¹ and Synthesized by the white adipose tissue specifically by differentiated adipocytes. It regulates the body weight, modulates the insulin activity, sensitivity, metabolism and reproductive functions. The diverse role of adiponectin and leptin in development of pathophysiology of T2DM still needs to be studied. There is need to study role of association of adiponectin & leptin with insulin resistance in T2DM subjects of Western UP State, India.

2. Materials and Methods

The present observational case control hospital based study was carried out after approval of Ethical committee of Swami Vivekanand Subharti University, Subharti Medical college in Collaboration With Department of Biochemistry and Department of Medicine on T2DM Patients & age-sex matched healthy adult population. A total of 120 newly detected established T2DM patients and 120 healthy control subjects were enrolled in the study as per inclusion criteria who visited/referred to medicine OPD. Informed consent was duly taken from each subject during the study period.

2.1. Inclusion criteria

1. Patient of either sex aged between 30- 60 years.
2. 120 recent onset (<2 years of disease duration) Type-2 diabetic patients

3. Subjects who are permanent resident of study area and willing to participate in present study.

2.2. Exclusion criteria

1. Subjects with chronic illness.
2. Subjects / Patient on insulin therapy or on oral hypoglycemic agents.
3. Migrating population.

2.3. Sample collection

Overnight fasting 4-5 ml of venous blood was collected from antecubital vein of each subject and dispensed 1.5-2 ml of blood in a fluoride-oxalate vial for blood glucose test and rest stored in a plain vial (3ml) for serum insulin, adiponectin and leptin investigation. The routine investigations were undertaken on the same day and sample was centrifuged at 3000 rpm for 10 minutes. The serum collected was immediately stored and labeled in aliquots at -80°C for investigation of adiponectin, leptin and insulin etc. respectively.

2.4. Anthropometrics indices

The anthropometric indices, waist circumference (WC) in centimeters, waist hip ratio (WHR), and body mass index (BMI) in kg/m² were noted in pre-tested study proforma.

2.5. Serum glucose estimation

Fasting glucose estimated by Glucose Oxidase Peroxidase (GOD-OPD) method on Siemens Auto-analyzer by processing internal as well as external quality control.

2.6. Insulin estimation

The Serum insulin was estimated by sandwich ELISA assay method using kits manufactured by M/S Epite Diagnostics, Inc, USA (human) insulin assay kit for research purpose by split sample assay & quality control sample supplied by manufacturer was processed along with study samples.

2.7. Insulin resistance

It was calculated using HOMA-IR (Homeostasis model assessment of insulin resistance) calculation.

$$\text{HOMA-IR} = \frac{\text{Fasting plasma insulin } (\mu\text{U/L}) \times \text{fasting glucose (mg/dl)}}{22.5}$$

2.8. Adiponectin estimation

The level of Adiponectin was assayed using Assaymax human adiponectin ELISA (sandwich) kit for research purpose. The quality control samples were supplied along with kit by manufacturer processed during analysis.

2.9. Leptin estimation

The level of leptin assay was estimated by using Diagnostics Biochem Canada Inc. kit (sandwich ELISA) for research purpose using QC samples provided by manufacturer.

2.10. Statistical analysis

Collected Data were analyzed using the Statistical Package of the Social Sciences (SPSS version 16.0). Data were presented as mean $\pm$ standard deviation. Independent sample t-test, Chi-square test, Pearson correlations were used to compare different parameters. The differences among the means (Mean $\pm$ SD) were considered significant if $P < 0.01$ & 0.05 .

3. Results

Present study conducted in 120 healthy populations, 69 male and 51 female, while out of 120 T2DM patients 63 were males and 57 were female in the age group of 30-60 years. Total number of subject in case group with BMI >25 kg/m² were 85 and in control group 30. In Asian Indian population BMI >23 kg/m² is considered obese. In the study we found non-significant but negative correlation of adiponectin with BMI, WHR, WC and HC. A strong negative correlation was found between adiponectin and WHR with statistically significant ($P=0.002$). Adiponectin levels in case was 7.70 ± 2.86 μ g/ml (Table 2) however it has a negative (non-significant) relation with fasting insulin, post-prandial insulin, post-prandial blood sugar and HOMA-IR respectively. There is significant positive correlation between adiponectin and HDL-Cholesterol ($P=0.014$) in study group (Table 1) however, it doesn't show any significant negative correlation.

In present study, leptin 36.35 ± 25.86 ng/ml (Table 2) correlated significantly with BMI ($P=0.0000$), WC ($P=0.007$), and HC ($P=0.0000$) (Table 1) respectively. The parameter of insulin resistance leptin showed significant positive correlation with fasting insulin ($P=0.002$), present study highlight significant positive correlation with triglycerides ($P=0.038$), strong negative correlation with HDL-Cholesterol ($P=0.017$) (Table 1) however it does not show any correlation with other parameters.

Table 2 shows that Demographic, Anthropometric & Biochemical parameters were found significant in study group than in control group $P < 0.001$.

The serum Insulin, HOMA-IR, & Leptin level were significantly higher in case group than in control group while adiponectin level were found to be low in case group than in control group.

The strength of correlation is not dependent on the direction or the sign. Thus $r = -0.172$ and $r = 0.172$ are equal in degree of association of measured variables. A positive correlation coefficient indicates that an increase in the first variable would correspond to an increase in the second

variable, Thus implying the direct relationship between the variables. A negative correlation indicates that an inverse relationship whereas one variable increases, second variable decreases. BMI (kg/m²), Waist circumference (cm) & Waist-hip ratio are shows the significant correlation with Adiponectin. Waist circumference (cm), Hip circumference (cm), Waist-hip ratio, Fasting insulin (mIU/L), HOMA-IR, Total Cholesterol (mg/dl), HDL (mg/dl) & LDL (mg/dl) highlights the significant correlation with Leptin.

4. Discussion

In present study in both the diabetic & non-diabetic group, we found that male subjects were dominant than female for T2DM. In the present study, the mean age for cases was 42.98 ± 8.16 years and controls was 36.75 ± 5.84 years which was in accordance with a study done by Diwan et al.¹² The present study revealed that mean BMI for control group was 22.56 ± 4.13 and T2DM (Study group) was 25.85 ± 4.34 . These findings were similar to the study reported by Lalrindiki et al.¹³

Present study reports that mean Waist Circumference (WC) in T2DM group were more i.e. 99.51 ± 3.65 cm than in control group i.e. 86.74 ± 8.09 cm which was in accordance to the study reported by Aurelian Bidulescu et al.¹⁴ and contradictory to the study done by Lalrindiki et al.¹³ Similarly our study revealed that mean waist hip ratio (WHR) were increased in T2DM group than in control group which is almost similar to the findings of Lalrindiki et al.¹³ Increased Waist circumference (WC) and Waist hip ratio (WHR) in T2DM group could be attributed to the fact that such individuals may have accumulated fat levels.

Mean Serum adiponectin level and mean serum leptin levels were compared with the incidence of impaired blood glucose level in the case study population. The study report revealed that adiponectin level were significantly lower ($P < 0.001$) in patient with increased Fasting Sugar and Post Prandial Sugar while Leptin level were significantly higher ($P < 0.001$) in patient with increased Fasting Sugar and Post Prandial Sugar which was in accordance with a study done reported by Diwan et al.¹² & Rajmangal choudhary et al.¹⁰

Present study results compared with Serum adiponectin and leptin levels with the incidence of dyslipidemia in the case study population we found that Total Cholesterol, Triglycerides, LDL, VLDL were significantly elevated while HDL were significantly lower in diabetic group. Decreased adiponectin and increased leptin levels with dyslipidemia in diabetic patient could be one the major reason for increased incidence of Coronary Heart Disease (CHD). Similarly our study results revealed that serum insulin and HOMA-IR were increased in T2DM. These findings were similar to the study reported by Aurelian Bidulescu et al.¹⁴

Present study results highlights that Adiponectin is positively correlated with HDL, TC, and LDL while other anthropometric indices markers of insulin resistance

Table 1: T2DM patients with demographic, anthropometric & biochemical parameters of study subjects

Parameters	Healthy Control (n=120)	T2DM (n=120)	P-Value
Age (Years)	36.75±5.84	42.98±8.16	0.020*
BMI (kg/m ²)	22.56± 4.13	25.85±4.34	0.001***
WC (cm)	86.74±8.09	99.51±3.65	0.001***
WHR	0.83±0.04	0.99±0.04	0.001***
Fasting Sugar(mg/dl)	86.85±11.53	158.74±37.83	0.001***
PP Sugar(mg/dl)	108.42±14.82	264.60±53.50	0.001***
Systolic Blood pressure mmHg	108.40±8.11	121.63±11.32	0.001***
Diastolic Blood pressure mmHg	84.6±7.86	88.5±10.71	0.001***
Cholesterol (mg/dl)	165.23±21.31	229.47±33.31	0.001***
TG (mg/dl)	126.83±21.60	243.05±40.181	0.001***
HDL (mg/dl)	45.48±9.06	34.34±6.91	0.001***
LDL (mg/dl)	99.27±12.47	146.67±32.67	0.001***
VLDL (mg/dl)	25.38±7.21	48.72±7.74	0.001***

Values are expressed as Mean ± Standard Deviation,

*Significant considered as *P<0.05, **P<0.01 ***P<0.001

M: Male, F: Female, BMI: Body Mass Index, WC: Waist Circumference, WHR: Waist Hip Ratio

Table 2: Level of Insulin, HOMA-IR, Adiponectin & Leptin in control and T2DM group

Variables	Healthy Control (n=120)	T2DM (n=120)	p-Value
Insulin (mIU/L)	6.41±0.99	13.23±2.09	0.001***
HOMA-IR	1.29±0.32	5.02±1.06	0.001***
Adiponectin (μg/ml)	27.18±5.02	7.70±2.86	0.001***
Leptin (ng/ml)	19.80±7.51	36.35±25.86	0.001***

*P<0.05, **P<0.01, ***P<0.001

Table 3: Pearson correlation studied of adiponectin and leptin with an anthropometric indices marker of insulin resistance and lipid profile

Parameters	Adiponectin		Leptin	
	r value	p value	r value	p value
BMI (kg/m ²)	-0.294	0.001	0.029	0.826
Waist circumference(cm)	-0.196	0.001	0.413	0.001
Hip circumference (cm)	-0.027	0.815	-0.433	0.001
Waist-hip ratio	-0.143	0.009	0.301	0.000
Fasting insulin (mIU/L)	-0.059	0.682	0.336	0.002
Fasting blood sugar (mg/dl)	-0.031	0.736	0.171	0.365
HOMA-IR	-0.055	0.702	0.280	0.002
Total Cholesterol (mg/dl)	0.059	0.522	0.393	0.002
Triglycerides (mg/dl)	-0.172	0.055	0.173	0.181
HDL (mg/dl)	0.071	0.440	-0.291	0.021
LDL (mg/dl)	0.102	0.264	0.313	0.014

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

and Triglycerides had negative correlation which is in accordance with the study reported by Prema Adhikari et al.¹⁵ Similarly, leptin is negatively correlated with HDL while other anthropometric indices markers of insulin resistance and Triglycerides had positive correlation similar to the study reported by Amita yadav et al.¹⁶

5. Conclusion

Present study highlights that adiponectin and leptin concentrations vary between T2DM and control participants. Low serum adiponectin and high serum leptin levels are associated with increased risk of T2DM, Increased WHR, WC and dyslipidemia. Increased leptin may promote while decreased adiponectin prevent generation of insulin resistance. Dyslipidemia could be a new component of insulin resistance syndrome. Adiponectin and Leptin can

be used as a useful biomarker for diagnosis and early identification of metabolic syndrome. In the future, these adipocytokines may also be used to prevent or to treat T2DM and its complications as prognostic marker.

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7. Conflicts of Interest

None.

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