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Association of leptin with insulin resistance in type 2 diabetes mellitus: A prospective study

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***Abstract*---**Introduction: Type 2 Diabetes mellitus (T2DM) is a chronic disease that is characterized by impaired glucose metabolism and insulin resistance. Leptin is a 16-kDa protein hormone, which is secreted by adipocytes. Plasma Leptin concentration increases in proportion to body fat mass and regulate food intake and energy expenditure to maintain body fat stores. Leptin binds with a leptin receptor (LEPR) that is located on pancreatic beta cells to regulate insulin secretion. Materials and Methods: This is a prospective and case-control study was conducted in the Department of Biochemistry at Great Eastern Medical School and hospital over a period of 1 year. After the inclusion of participants in the study, their demographics such as age, BMI, gender, and smoking history were noted in self-structured questionnaires. Their blood was drawn and sent to the laboratory for Lipid profile levels, insulin resistance and leptin levels. The serum levels of Leptin were measured using a radioimmunoassay (RIA). Results: The probable association between Leptin and Insulin resistance in type 2 diabetes mellitus. 60 recent onset (<5 years) diabetics and age-sex matched 60 non-diabetic controls were assessed for physical and chemical parameters. All the physical parameters showed positive correlation with Leptin and the HOMA-IR score, the strength of association being highest between insulin resistance and abdominal circumference. Leptin and Insulin resistance showed no

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correlation. Findings were lower in controls. Conclusion: In our study, significant higher level of Leptin was found in insulin resistant subjects compared to the subjects without the condition in both genders. This finding provides an insight into the explanation why the metabolic risk was different among persons with same degree of adiposity and may help identify the people at risk for diabetes and/or cardiovascular diseases across adiposity level and thereby an important contribution in clinical and preventive measures.

***Keywords*---**diabetes mellitus, insulin resistance, leptin, HOMA-IR.

Introduction

Type 2 Diabetes mellitus (T2DM) is a chronic disease that is characterized by impaired glucose metabolism owing to insulin resistance. [1] Leptin is an important adipokine hormone, which is essential in carbohydrate metabolism and affects insulin sensitivity and hence, Diabetes mellitus. Leptin has been reported to be involved in the pathogenesis of Diabetes. It has been determined that Leptin reduces Insulin synthesis and secretion and increases hepatic extraction of insulin. It has also been determined that Leptin increases insulin sensitivity. [2]

Leptin binds with a leptin receptor (LEPR) that is located on pancreatic beta cells to regulate insulin secretion. [3] Insulin resistance is essential for the development of T2DM. Clinical study reports suggest that elevation of Leptin levels occurs owing to the upregulation of the Leptin gene owing to insulin resistance and hyperinsulinemia. [4] It has been reported that Leptin affects whole-body insulin sensitivity by regulating insulin-mediated glucose metabolism by skeletal muscle as well as hepatic regulation of gluconeogenesis. [5]

Leptin has been determined to have an inhibitory effect on insulin secretion. [6] In obesity, a decreased sensitivity to leptin occurs (similar to insulin resistance in type 2 diabetes), resulting in an inability to detect satiety despite high energy stores and high levels of leptin. Although leptin reduces appetite as a circulating signal, obese individuals generally exhibit a higher circulating concentration of leptin than normal weight individual's due to their higher percentage body fat. These people show resistance to leptin, similar to resistance of insulin in type 2 diabetes, with the elevated levels failing to control hunger and modulate their weight. [7]

Leptin can improve insulin resistance or conversely it may induce insulin resistance. Leptin and Insulin have been reported to have antagonistic actions. Thus, the expression of Leptin may considerably affect the pathogenesis of diabetes. **[8]** There are not many studies that establish the role of leptin and polymorphism of its genes in the pathogenesis of T2DM. Few studies have reported that diabetes mellitus is not associated with Leptin levels [9] whereas some studies have reported a significant positive association between plasma Leptin levels and diabetes. [10] However, contradictory reports are also

available. [4] Some studies have established the association between plasma Leptin levels and diabetes mellitus only in men. [11] Previous studies examining the association between serum Leptin levels and diabetes mellitus were restricted to specific racial/ethnic groups and were not consistent in their findings. [12-15]

Material and Methods

This is a prospective and case-control study was conducted in the Department of Biochemistry at Great Eastern Medical School and hospital over a period of 1 year.

Inclusion criteria

60 either gender Type-2 diabetics of 30- 60 years of age as a Case group. 60 either gender non-diabetic healthy of 30- 60 years of age as a Control group.

Exclusion criteria

Subjects with any comorbidities such as Hypertension, previous history of cardiovascular events were excluded from the study. Patient on Insulin therapy or on oral hypoglycaemic other than short acting 2nd generation sulfonylurea was also excluded. After the inclusion of participants in the study, their demographics such as age, BMI, gender, and smoking history were noted in self-structured questionnaires. Their blood was drawn and sent to the laboratory for Lipid profile, Insulin resistance and Leptin levels. The serum levels of Leptin were measured using a Invitrogen Human Leptin Elisa kit by Thermofisher Scientific according to the manufacturer's instructions (normal range: 2.5-21.8 ng/mL). [15]

Fasting 10 ml venous blood samples were obtained from all participants and collected into Sodium fluoride and plain vacutainer. Unique ID number was given to each sample to hide the identity of all participants. All samples were centrifuged at 3000 RPM for a period of 10 minutes to obtain plasma and serum. Blood Glucose (FBS) measured by GOD POD method and Lipid profile (S. Cholesterol, Triglyceride, HDL, VLDL, LDL) measured by semi auto analyzer ERBA Chem 7, using ERBA Mannheim kits from all samples. Fasting Insulin level estimation was done by Enzyme linked immuno sorbent assay (ELISA) method based on electrochemiluminiscence and HOMA-IR was calculated multiplying fasting serum Insulin (IRI) mIU/L with fasting blood glucose (FPG) mg/dl from the same sample then dividing by the constant 405, i.e. HOMA-IR = (FSIxFPG)/405.

Statistical analysis

Statistical analysis was done using SPSS, v. 25.0. Continuous variables including Age, Insulin resistance, Body mass index, Cholesterol levels and Leptin levels were analyzed via descriptive statistics and were presented as means and Standard Deviation. T-test and Chi-square were applied as appropriate. P-value of less than 0.05 meant that the difference between the groups is significant.

Result

Total 60 type 2 diabetics (<5 years disease duration) and 60 age sex matched non-diabetic controls were studied. Average age, male: female ratio and mean values of different physical and bio-chemical parameters among them are summarized in Tables 1 & 2 respectively.

Table 1: Physical parameters of study & control group

Parameters	Study Group (n=60)	Control group (n=60)	p-value
Age (years)	46.37±5.53	44.5±5.19	0.4127
Male: Female	37:23	39:21	
Total body weight (kg)	74.85±6.22	64.34±5.85	0.0001
BMI (Weight in kg/ Height in meter^2)	31.84±3.95	28.19±2.08	0.04
Abdominal Circumference (cm)	109.11±0.88	104.16±6.28	0.0001
Waist/Hip ratio	1.85±0.09	1.02±0.06	0.0001

Table 2: Bio-chemical parameters of study & control group

Parameters	Study group (n=60)	Control group (n= 60)	P value
Fasting blood glucose (mg/dl)	157.07±16.46	79.16±8.28	0.0001
HOMA-IR Score	2.36±2.96	1.48±0.94	0.003
Leptin (ng/ml)	16.67±2.43	9.67±3.97	0.001
HbA1c %	6.92±1.43	5.97±3.97	0.042

Table 3: Bio-chemical parameters of study & control group

Parameters	Study group (n=60)	Control group (n= 60)	P-value
Total Cholesterol (mg/dL)	188.3 ± 19.3	168.2 ± 18.2	0.0321
Serum Triglycerides (mg/dL)	174.27 ±15.19	151.39 ±14.69	0.0024
HDL-C (mg/dL)	42.37 ±5.71	48.61 ±5.45	0.0043
LDL (mg/dL)	111.08 ±10.56	89.32 ±9.79	0.0036
VLDL (mg/dL)	34.85 ±3.03	30.27 ±2.93	0.0108

Table 4. Pearson's correlation analysis of leptin with other parameters

	Correlation coefficient r value	p-value
Total body weight (kg)	0.32	P<0.05
BMI (Weight in kg/ Height in meter^2)	0.84	P<0.05
Abdominal Circumference (cm)	0.63	P<0.05

Significant positive correlations were found between Leptin and total body weight (r=0.32, P<0.05), BMI (r=0.84, P<0.05), abdominal circumference (r=0.63, P<0.05), but correlation between Leptin and HOMA-IR score was not significant.

Table 5. Pearson's correlation analysis of HOMA-IR with other parameters

	Correlation coefficient r value	p-value
Total body weight (kg)	0.28	P<0.05
BMI (Weight in kg/ Height in meter^2)	0.63	P<0.05
Abdominal Circumference (cm)	0.53	P<0.05
Waist/Hip ratio	0.37	P<0.05

Physical parameters showed significant positive correlations with HOMA-IR score with coefficients of correlation as follows: Total body weight (r=0.28, P<0.05), BMI (r=0.63, P<0.05) Abdominal circumference (r= 0.53 P<0.05) and waist/hip ratio (r=0.37, P<0.05). Among the physical parameters strength of association was found highest between abdominal circumference and HOMA-IR score. All the physical parameters for obesity and chemical parameters like HOMA-IR score and Levels of leptin were found to be significantly lower in non-diabetic controls than in diabetics.

Discussion

Diabetes is increasing steadily all over the world. The International Diabetes Federation estimates that more than 382 million people have diabetes globally and this number is expected to exceed 590 million by 2035 [15]. Growing evidence suggests that, in addition to the regulation of energy homeostasis, the adiposity hormone leptin also plays a key role in glucose metabolism. [16] Leptin is a multiple-function adipocytokine involved in the regulation of food intake, energy storage and saccharide and lipid metabolism. Impaired regulation of food intake in consequence of leptin resistance is presented in connection with the aetiopathogenesis of obesity and insulin resistance, but its role in development of these diseases is still not clear. [17] Our study revealed a positive correlation between leptin and adiposity. This has been supported by various previous studies. [18]

Being the product of the obese secreted from adipose tissue Leptin signals the amount of energy stores to the brain and is implicated in the regulation of food intake and energy balance. Hyperleptinemia in obesity may be due to leptin resistance, which may arise from impaired leptin transport across the blood-brain barrier (BBB), defects in leptin receptor signaling, and blockades in downstream neuronal circuitries. [19] Being supported by all these, our study emphasizes the potential of leptin to be used as a marker for obesity. Present study also reveals a significant positive correlation among the physical parameters of obesity and degree of insulin resistance (HOMA-IR score). Further the strength of association was found to be highest between abdominal circumference and insulin resistance. This suggests a probable role of obesity mainly the central or visceral type in the pathogenesis of insulin resistance. Finding is corroborative with some previous

studies that also found direct correlation between central obesity, insulin resistance and cardiovascular risk factors. [20]

Regarding the adverse effects of obesity in particular the visceral obesity on glucose metabolism many probable mechanisms have been suggested which include, excessive lipid "supply" by a mechanism currently referred to as "lip toxicity." When FFA (Free Fatty Acids) is elevated for a prolonged period, they have a direct effect on insulin action in skeletal muscle tissue and liver, reducing the normal responses to insulin to promote glucose uptake and to suppress hepatic glucose output, respectively. In both of these tissues, FFA increase cellular levels of acyl-CoA derivatives, which lead to an increase in the activity of cellular signaling molecules, termed serine kinases that oppose the normal tyrosine phosphorylation cascade of the insulin receptor. [21]

The increased intracellular lipid accumulation that occurs in obese subjects as "ectopic fat", that is, triglyceride stored in the target organs themselves rather than in a benign adipose depot is an important source of intracellular acyl-CoA molecules that can affect normal insulin signal transduction. Other proteins secreted by adipose tissue, including the important inflammatory mediators like Interleukin-6 (IL-6) and Tumor necrosis factor-(TNF), may have adverse effects on energy metabolism and insulin sensitivity in liver and muscle and play key roles in the development of insulin resistance in obesity.[22] Revolutionary concepts is that adipose tissue is not only a simple reservoir for energy stored as triglycerides but also serves as an active secretary organ, releasing many peptides, complement factors, and cytokines into the circulation called adipocytokines. Most important of this class so far are Leptin, Adiponectin, Resistin etc. Complex interactions between these mediators are proposed to be involved in the causation of insulin resistance. [23]

Many of them have shown that exogenous Leptin may improve the state of glucose metabolism and insulin sensitivity especially in lipodystrophy subjects. [24] Based on all such previous data and our present findings it seems that, though obesity is a definite precondition in insulin resistance, single adipocytokine like Leptin may not be solely responsible for its causation. [25]

Conclusion

Our study concluded that Leptin is a novel marker for obesity, though it is there is probable association between leptin and Obesity but none between leptin and Insulin resistance. Physical parameters especially abdominal circumference was found to have significant positive correlation with insulin resistance. Therefore, it seems that, obesity mainly central type may lead to insulin resistance and thereby diabetes mellitus. Leptin, though an obesity marker, is not significantly correlated with insulin resistance alone, which may point towards the multifactor causation of insulin resistance in type 2 diabetes mellitus.

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