Evaluation of Leptin as a Marker of Insulin Resistance in Type 2 Diabetes Mellitus

Abstract

Background: Type 2 diabetes mellitus (T2DM) is a leading cause of mortality and morbidity worldwide, whose incidence is rapidly increasing in India. T2DM is caused by varying degrees of insulin resistance (IR) and relative insulin deficiency. Leptin, an adipokine with the primary function of regulating energy balance, is found to mediate insulin secretion and sensitivity in peripheral tissues. Hence, we aimed to determine the role of leptin in the development of IR in newly diagnosed T2DM patients. Aim: This study aims to compare the leptin levels and homeostatic model assessment (HOMA-IR) levels in the study population. Material and Methods: The study included a total of sixty patients newly diagnosed with T2DM. Their fasting blood samples were collected to estimate the glucose, insulin, and leptin levels. IR was calculated using HOMA-IR formula. Statistical analysis was done by Pearson’s correlation, Spearman’s correlation, and One-sample Wilcoxon Signed Rank test. Results: Leptin and HOMA-IR levels were significantly high in T2DM patients (P < 0.001) when compared with reference values. Body mass index showed a significant positive correlations with insulin (r = 0.40, P < 0.01), HOMA-IR (r = 0.37, P < 0.01), and leptin levels (r = 0.90, P < 0.01). Leptin levels showed significant positive correlations with plasma insulin (r = 0.35, P < 0.01) and HOMA-IR levels (r = 0.31, P < 0.05). The correlation between leptin and HOMA-IR levels was more pronounced and significant among the obese T2DM subjects (r = 0.82, P = 0.01). Conclusion: Hyperleptinemia reflecting lepbin resistance plays an important role in the development of IR in obese T2DM patients, making leptin a possible biomarker for the same.

Keywords: Homeostatic model assessment-insulin resistance, insulin resistance, leptin, type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia over a prolonged period. As of 2014, an estimated 387 million people have diabetes worldwide, of which type 2 DM (T2DM) makes up about 90% constituting about 8.3% of the total adult population. Insulin resistance (IR) is a condition in which cells fail to respond to the normal actions of the hormone insulin, despite its normal secretion. The resultant effects are impaired glucose utilization by the cells, leading to hyperglycemia. IR along with decreased insulin secretion results in T2DM. Homeostatic Model Assessment (HOMA) is a method used to quantify IR.

Leptin is an adipocyte-derived hormone, which is also known as an adipocytokine as it plays a role in the inflammatory process involving adipose tissues. It has a diverse array of functions, of which the most important one is to regulate energy balance. It does so by decreasing the appetite or energy intake and by increasing energy expenditure. Hence, it is rightly called as the “satiety hormone.” By regulating energy metabolism, it also regulates the body fat stores. Hence, the leptin levels in the body correspond to the adipose tissue mass. More recently, leptin was found to mediate the secretion and peripheral tissue sensitivity of the hormone insulin. The role of leptin in altering glucose metabolism and insulin sensitivity in T2DM are not fully explored. Since IR is the basis behind the development of T2DM, we designed the present study to estimate the levels of leptin and IR (using HOMA-IR) in newly diagnosed T2DM patients, in a view to determine the role of leptin in the development of IR in T2DM.

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Materials and Methods

The study is a descriptive study, done between March 2013 and March 2015. The study was approved by Institute Research Committee and Institute Ethics Committee (IEC reference number: RC/13/01).

The required sample size was estimated to be 59 based on the following formula:

\[
 n = \frac{Z^2_{1-\alpha/2} \times \sigma^2}{d^2}
\]

Where standard deviation (\(\sigma\)) was 19.5, absolute precision (\(d\)) was 5, and desired confidence level (\(Z^2_{1-\alpha/2}\)) was 95%.

Inclusion criteria

A total of 60 patients newly diagnosed as T2DM (as per ADA criteria) and between 30 and 50 years age group were included in the study.

Exclusion criteria

Patients on steroid therapy, known case of Cushing’s disease, Addison’s disease, hypertension, coronary artery disease, stroke, polycystic ovary syndrome, and dyslipidemia were excluded from the study. After an overnight fast, blood samples were collected from the patients, centrifuged and the serum obtained was used for the following analysis. Glucose was estimated spectrophotometrically by Hexokinase method in Cobas Integra 400 plus from Roche diagnostics, insulin was estimated by sandwich electrochemiluminescence immunoassay (ECLIA) method in Cobas e411 from Roche Diagnostics, Leptin was estimated by sandwich ELISA method by dbc human Leptin Elisa kit. IR was calculated using HOMA-IR by the following formula:

\[
\text{HOMA-IR} = \frac{(\text{Fasting glucose [mg/dl]} \times \text{Fasting Insulin [mIU/L]})}{405}
\]

HOMA-IR levels >2 was considered as IR.\[^8\]

Statistical methods

Data were entered into MS Excel 2007 and analyzed by SPSS for Windows (version 20.0, Armonk, New York, IBM Corporation). Descriptive statistics such as numbers, percentages, mean and standard deviation were used. One-sample Wilcoxon Signed Rank \(t\)-test was used to compare the biochemical values of the study population with their reference values. Pearson’s correlation was used to correlate glycated hemoglobin with plasma insulin, HOMA-IR, and plasma leptin levels. Spearman’s correlation was used to correlate plasma leptin levels with plasma insulin and HOMA-IR. \(P < 0.05\) was considered as statistically significant.

Results

The study was done on 60 newly diagnosed T2DM patients. It included 34 males and 26 females. The baseline parameters of the study group are illustrated in Table 1.

The mean body mass index (BMI) of the study population was 26.9 ± 3.5 kg/m\(^2\). Based on their BMI, the subjects were categorized as normal (\(n = 18\), overweight (\(n = 30\)), and obese (\(n = 12\)). One-sample Wilcoxon Signed Rank \(t\)-test was used to compare the median of leptin and HOMA-IR levels of T2DM with reference values. There was a significant elevation in the median levels of HOMA-IR and leptin (both males and females) in the T2DM patients than the normal reference median levels [Table 2]. BMI showed a highly significant and positive correlation with plasma insulin \((r = 0.294, P = 0.002)\) and HOMA-IR levels \((r = 0.327, P = 0.04)\) in T2DM patients. BMI showed a highly significant and positive correlation with plasma leptin levels \((r = 0.90, P < 0.001)\) in T2DM patients [Figure 1]. In T2DM patients, the plasma leptin levels showed a positive correlation with plasma

| Table 1: Baseline parameters of the study population of type 2 diabetes mellitus patients \((n=60)\) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | FBS (mg/dL)     | PPBS (mg/dL)    | HbA1c%          | Plasma insulin (mIU/ml) | HOMA-IR       | Plasma leptin (ng/mL) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Mean            | 160             | 279             | 8.1             | 24.7            | 9.8             | 21.3            | 44.9            |
| SD              | 52              | 64              | 1.2             | 19.3            | 7.8             | 28.5            | 36.3            |
| Median          | 145             | 263             | 7.9             | 19.6            | 7.3             | 8.1             | 38.5            |

SD: Standard deviation; FBS: Fasting blood sugar; PPBS: Postprandial blood sugar; HbA1c: Glycated hemoglobin; HOMA-IR: Homeostatic model assessment-insulin resistance
insulin levels ($r = 0.35, P = 0.007$). The plasma leptin levels also showed a significant positive correlation with HOMA-IR levels in T2DM patients ($r = 0.31, P = 0.015$) [Figure 2]. The degree of correlation increased with increase in BMI, which was more significant in the cases of obese T2DM subjects ($r = 0.82, P = 0.01$) [Figure 3].

**Discussion**

Most of our patients had BMI levels above the normal, with 50% of them being overweight and another 20% of them being obese, emphasizing that obesity is a risk factor for developing T2DM.[10] Using the HOMA-IR formula, we found that 82% of our patients had already developed IR with HOMA-IR levels $>2$, which is a contributing factor for the development of T2DM.[11] The majority of the study population had high leptin levels (70%), irrespective of their gender differences. This accounts to their higher BMI levels, as the leptin levels in general increase proportionately with body fat mass.[8] The BMI of the study population showed significant positive correlations with their insulin, HOMA-IR, and leptin levels which were in agreement with the findings of the studies done by Chung et al. and Okita et al.[12,13] The positive correlation between leptin and BMI were also established in a study done by Das et al.[14] The median HOMA-IR and leptin levels (in both genders) of the study population were significantly higher which was in agreement with a study done by Jayagopal et al. and Boyko et al.[15,16] Similar results were established by Das et al.[14] Mohiti et al. stated that it was obesity leading to increased insulin and leptin levels, resulting in IR and leptin resistance found in T2DM.[17] This theory is also supported by Wang et al., who deduced that there is a simultaneous occurrence of IR and leptin resistance in obesity.[18] Apart from this obesity risk factor, the high levels of leptin in the present study population could also be due to their genetic predispositions, as demonstrated by Mente et al. who compared the adipokine profile of South Asians with Chinese, Canadians and Aboriginals, and found that the South Asians had the least favorable adipokine profile.[19] A study by Liao et al. suggested that the early onset of T2DM in the Han Chinese population of Taiwan was due to their leptin receptor gene polymorphisms, which in turn lead to leptin resistance and elevated leptin levels to overcome the resistance.[20] Wannamethee et al. found that the risk of developing T2DM increase with increasing leptin levels.[21] Hence, the results of the present study are consistent with these studies on leptin levels and IR in T2DM. The leptin levels of the T2DM patients showed significant positive correlations with their insulin. The correlation between leptin and HOMA-IR levels was more pronounced and significant in the obese T2DM subjects. In agreement with our findings, studies by Das et al. and Yadav et al. also showed a positive correlation between leptin and HOMA-IR levels in T2DM patients.[14,22] On the contrary, Mahadik et al. disagreed with any such correlation between leptin in HOMA-IR levels in T2DM patients.[23] Mohiti et al. observed in their study that leptin and HOMA-IR showed positive correlation only in the obese T2DM patients, while they had a negative correlation.

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**Table 2: Comparison of leptin levels, homeostatic model assessment-insulin resistance levels of type 2 diabetes mellitus patients (n=60) with reference levels**

| Study population | Median Plasmaleptin (ng/mL) | Reference Median | P value |
|------------------|-----------------------------|------------------|--------|
| Males            | 8.1                         | 3.8              | <0.001*|
| Females          | 38.5                        | 7.4              | <0.001*|
| HOMA-IR          | 7.3                         | 1                | <0.001*|

*P<0.01 - highly significant. HOMA-IR: Homeostatic model assessment-insulin resistance

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**Figure 2: Correlation of plasma leptin and homeostatic model assessment-insulin resistance levels in type 2 diabetes mellitus patients**

**Figure 3: Body mass index-based correlation of plasma leptin and homeostatic model assessment-insulin resistance levels in type 2 diabetes mellitus patients**
in case of nonobese T2DM patients. Thus, obesity is the major pathogenic factor common to both leptin resistance and IR. The correlations of leptin levels with insulin, and HOMA-IR levels surely indicate the functional link between these two hormones. Leptin has an inhibitory effect on the insulin secretion, which is due to the leptin-induced proinflammatory cytokines such as C-reactive protein and Interleukin-6, causing apoptosis of pancreatic β-cells. In compliance to this theory, leptin levels should have an only negative correlation with insulin levels, which is opposite to what we see in the present study. This can be explained by the underlying IR leading to elevated insulin levels, along with a possible leptin resistance in our study population. The possible explanation could be because leptin and insulin share the same pathway for their actions, leading to a leptin-insulin crosstalk. Sun and Rutter described that the central appetite controlling actions of leptin and insulin are mediated through the hypothalamic AMP-Kinase pathway. Other mechanisms for the leptin-mediated insulin sensitivity are by inhibiting the Sterol regulatory element binding protein-1 and stearoyl-CoA desaturase-1. Leptin also stimulates the insulin receptor substrate and PI-3K in the insulin signaling pathway, thus increasing the peripheral insulin sensitivity by way of increased glucose uptake and fatty acid oxidation in the tissues. Leptin in the hypothalamus initially activates the JAK-STAT pathway, which in turn stimulates the PI-3K pathway of insulin, and this mechanism is known as the “leptin–insulin crosstalk.” Leptin resistance causing obesity which could be due to several factors such as mutations of the leptin receptor gene, mutations in the target neurons, decreased transport of leptin across the blood-brain barrier, defects in the postreceptor signaling pathway. Leptin resistance, which is more common with obesity, also leads to IR, linking leptin’s role in the pathogenesis of T2DM. Walsh et al. proposed that leptin is a predictor of T2DM risk in men. McNeely et al. also found in their study that higher leptin levels suggested an increased risk for T2DM. These are the possible mechanisms by which leptin levels correlate with IR in T2DM.

Conclusion

Hyperleptinemia reflecting leptin resistance plays an important role in the development of IR in T2DM patients, making leptin a possible biomarker for assessing IR levels in T2DM patients, especially in the obese.

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Conflicts of interest

There are no conflicts of interest.

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