
Leptin Level in Type I diabetic patients

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Abstract:

Background: Serum leptin levels reflect the amount of body fat. However several studies suggest that insulin may also regulate serum leptin levels, especially those newly diagnosed patient with type I diabetes (treated with insulin).

Aim of the study: The aim of the study was to test the leptin level in newly diagnosed children with type I diabetes and its level after institution of insulin therapy.

Subjects and Materials: Thirty children with new onset type I diabetes were studied. Serum leptin levels (ELIZA) were measured at presentation before insulin therapy was started and at 3 months of follow-up. Those patients were compared. with thirty healthy children match for age and body mass index.

Results: The results showed significantly low levels of serum leptin for the diabetic patients before insulin in comparison to its level for both diabetic patients (3 months of insulin therapy) and the control group. [(4.1 ± 0.5), (6.0 ± 0.4), (6.9 ± 0.2) ng/ml] P<0.005, 0.05 respectively). In addition to a significant increase in body wt. and BMI for the diabetic patients after insulin therapy (3 months) in comparison to their results before initiation of insulin therapy [(39.7 ± 3.1), (35.2 ± 2.9) kg], [(20.1 ± 0.8), (17.7 ± 0.4) kg/m²] (P<0.005, 0.05) respectively. The study shows also a positive correlation between serum leptin level and BMI for those patients after 3 months post treatment (r=0.6, p=0.04).

Conclusion: Serum leptin levels are low in newly diagnosed children with type I diabetes, after 3 months of insulin therapy circulating levels increase and became comparable to levels found in healthy control subjects. It remains to be determined whether this is solely due to insulinization or is also the result of nutritional replenishment and changes in fat cells metabolism.

Key words: Leptin, HbA1c, insulin dependent diabetes mellitus (Type 1).

Introduction:

Serum leptin levels reflect the amount of body fat, and because of the association of adiposity with insulinemia, leptin levels have been found to correlate positively with insulin levels.^(1,2,3) However, it is still controversial, whether insulin per se, independent of deposit, modulates plasma leptin levels. In vivo insulin infusion was found to be associated with increase in leptin levels in some^(4,5,6,7,8,9,10,11) but not all studies^(12,13,14,15,16). In vitro studies have shown that insulin stimulate adipocyte leptin production^(17,18,19). Studies in type 2 diabetes demonstrate a relationship between leptin and insulin regardless of adiposity^(20,21,22). In other studies of healthy children, insulin level correlated directly with leptin independently of body fat mass⁽⁷⁾.

Aim of the study:

The aim of the study was to test the leptin level in newly diagnosed children with type I and its level after institution of insulin therapy.

Patients with new-onset type I diabetes who are insulin deficient may present an ideal in vivo experimental set-up to assess the effect of insulin on leptin.

Subjects and Methods:

Subjects:

Thirty (30) children [sixteen (16) boys and fourteen (14) girls], aged (10.5 ± 0.6 yrs) were participated in our study, whom attended to National Diabetes Center (NDC), from (July 2008 to

December 2008). They were with new onset type I diabetes with classical symptoms. The patients required to subcutaneous insulin as the initial therapy and for 3 months duration of follow-up the patients.

The diabetic patients were compared with thirty (30) apparently healthy children after taking details of their medical history as control group. They were [seventeen (17) boys and thirteen (13) girls], aged (10.2 ± 0.4 yrs).

Methods:

Blood samples, five (5) ml for plasma blood glucose, serum leptin and hemoglobin A1C (%) assay, were collected at presentation before insulin therapy, was initiated, and after 3 months of follow-up. All samples were obtained after overnight fasting in control and patients.

Because we deal with children it is difficult to convince any parents to let their children been received insulin; therefore the results of control children were compared only with the data of the diabetic children before treating with insulin.

Two (2) ml was put in ethylene diamine tetracetic acid (EDTA) containing tube for HbA1c estimation. The remaining blood (3ml) was allowed to clot in plain tube at room temperature. The serum was aspirated after centrifugation at (3000 rpm) for 30 minutes divided in aliquots in plastic tubes and stored at (-20°) until the time of estimation.

For glucose estimation, plasma was used and determined by using kit (Biocom, Germany),

that based on the PAP enzymatic determination of glucose (Trinder 1969).

Serum leptin was estimated by using DRG leptin (sandwich) enzyme immunoassay kit (DRG) instruments Gmb H, Germany) (considine RV, Sinha MR, 1996).

HbA1c was determined by using the Bio-Rad VARIANT Hemoglobin A1c Program utilized principle of non ion-exchange high performance liquid chromatography (HPLC) for the automatic and accurate separation of HbA1c.

Body mass index(BMI) was calculated as the weight in kilogram (kg) per height in meter squared (m²).

$$\text{BMI (Kg/m}^2\text{)} = \text{weight (kg)} / \text{Height (m}^2\text{)}.$$

Results:

Sex (M/F), age, Ht, wt, and BMI (Mean $\pm$ SD) for both the control and diabetic patients groups (before insulin therapy and 3 months after treatment), were listed on table (.1).

Table (1) shows a significant increase in body wt. and BMI for those the diabetic patient after insulin therapy (3months) in comparison to their results before the institution of insulin therapy (P.005).

Serum leptin, plasma glucose, hemoglobin A1c % for both the control and diabetic patients group (before and 3 months after insulin therapy) were shown on (table 2).

The data showed low significant result of serum leptin level and high significant result of FPS and HbA1c levels for the diabetic patients before insulin therapy in comparison to its level for both the diabetic patient (3months of insulin treatment), and the control group (P<0.005, 0.05) respectively, (table 2). While such difference was not found between leptin level, FBS, and HbA1c for the diabetic patients (3 months of insulin therapy) and their levels for control group (table 2).

Furthermore, the increase in leptin level was correlated positively with BMI at 3 months post treatment with insulin.(r=0.6, P=0.04).

Table (1): Sex (M/F), age, Ht, wt, and BMI for control and patients.

Group		Sex (M/F)	Age (yr)	Ht. (m)	Wt. (kg)	BMI (Kg/m ²)
Control		14/11	10.2 $\pm$ 0.24	1.44 $\pm$ 0.20	36.6 $\pm$ 3.0	18.5 $\pm$ 0.2
Diabetes Mellitus patient	Before insulin	16/14	10.5 $\pm$ 0.6	1.40 $\pm$ 0.30	35.2 $\pm$ 2.9*	17.7 $\pm$ 0.4*
	After insulin	16/14	10.5 $\pm$ 0.6	1.41 $\pm$ 0.15	39.7 $\pm$ 3.10*	20.1 $\pm$ 0.8*

*P < 0.005

Table (2): serum leptin, FBS, and HbA1c %

Group		Serum leptin (ng/ml)	FBS (nmol/L)	HbA1c %
Control		6.9 $\pm$ 0.20 ²	4.83 $\pm$ 0.37 ²	6.1 $\pm$ 0.9 ²
Diabetes Mellitus patient	Before insulin	4.1 $\pm$ 0.5 ^{1,2}	12.50 $\pm$ 1.1 ^{1,2}	10.3 $\pm$ 0.7 ^{1,2}
	After insulin	6.0 $\pm$ 0.4 ¹	5.2 $\pm$ 0.2 ¹	6.9 $\pm$ 0.4 ¹

¹P < 0.005

²P < 0.05

Discussion:

The finding of this study suggested, that insulin may play a role in modulating leptin levels. When type I diabetic children are insulin deficient, which is classically the case at initial presentation, their leptin levels are significantly low to a control group. Furthermore, their leptin level does not correlate with BMI, indicating that factors besides adiposity are operational. Soon after the institution of insulin therapy leptin levels increase and become comparable to control group. These results suggest a stimulatory role of insulin on leptin production. On the other hand, nutritional depletion and the patients' catabolic state could potentially explain the low leptin levels at initial presentation.

Several studies have investigated the role of insulin on leptin in vivo. Although some of the studies showed elevations in leptin level after insulinemia^(4,5,6,7,8,9,10,11). Others reported no changes^(12,13,14,15,16). This discrepancy related to the different rates of insulin infusion and the different durations of insulin administration. In vitro studies also revealed a stimulatory role of insulin on leptin production in human and rat adipocytes^(6,17,18). In primary cultures of human abdominal adipocytes, insulin increased leptin gene expression at 72hr, followed by elevation in leptin concentrations in the culture medium⁽⁶⁾.

In human mammary fat cells, insulin stimulated a dose- dependent increase in leptin, whereas removal of insulin was followed by rapid decrease in leptin expression⁽¹⁷⁾. In rat adipocytes, insulin increased both the secretion and the production of leptin⁽¹⁸⁾.

In type 2 diabetes, hyperinsulinemia was associated with elevated leptin levels independent of body mass, suggest a stimulatory role of insulin on leptin production^(22,23,24). In adults with type I diabetes of long duration, leptin levels were higher than control values⁽²⁵⁾. This is most likely due to peripheral hyperinsulinemia resulting replacement modalities⁽²⁶⁾.

Conclusion: Serum leptin levels are low in newly diagnosed children with type I diabetes, after 3 months of insulin therapy circulating levels increase and became comparable to levels found in healthy control subjects. It remains to be determined whether this is solely due to insulinization or is also the result of nutritional replenishment and changes in fat cells metabolism.

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