Serum Leptin Levels in Children and Adolescents with Type 1 Diabetes Mellitus

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Abstract:

Background: There is a potential interaction between leptin and insulin; insulin may alters leptin secretion/action (or *vice versa*), dysregulations of this system could contribute to disease states such as diabetes.

Objective: To examine the effect of exogenous insulin on serum leptin in type1 diabetes patients, and determine the relation between serum leptin levels and other parameters including gender effect.

Patients and methods: The study was carried out at the National Diabetes Center (NDC)/ Al-Mustansiriya university on a total (77) subjects including (57) type 1 diabetes mellitus subjects formed of (15) patients who were newly diagnosed with type1 diabetes and (42) children and adolescents previously diagnosed with type1 diabetes mellitus who were on intensive insulin therapy for two years or more. Other (20) patient serves as a healthy control. All subjects were matched with age, and sex. The BMI was calculated as weight (kg)/height in meter square (m^2). Insulin dose was determined as dose/kg/day, after an overnight fasting blood was collected for laboratory evaluations consisted of measuring fasting serum glucose (FSG), glycated hemoglobin (HbA_{1c}), and lipid profile. Estimation of Leptin was examined at least 2h after insulin injection and meal ingestion which should not affect serum leptin concentration.

Results: the current study showed significant lower serum leptin level, and higher FBG in patients who were newly diagnosed with type1 diabetes before initiation of insulin therapy compared with (6-7) days of insulinization. On the other hand, the group of newly diagnosed patients had lower serum leptin levels, insulin dose, BMI, and higher FBG, HbA_{1c} versus patients on intensive insulin therapy. Moreover, they had comparable serum leptin levels and a higher serum FBG, HbA_{1c} compared to control group. As well as, group on intensive insulin therapy had higher serum leptin, BMI, FBG, HbA_{1c} compared to control group. On the other hand, there is no significant difference in the levels of total cholesterol (TC), and triglycerides (TG) among the studying group, The result showed that diabetic girls have higher serum leptin levels, BMI, and TG compared to diabetic boys, and there is no significant difference in the insulin doses between them, Finally, there is a significant positive correlation between leptin levels and all of the insulin dose, BMI, duration, and TG. Also, there is a positive correlation between insulin dose and BMI, and negative correlation between insulin dose and FBG in type1 diabetic patients.

Conclusion: This study suggests that insulin treatment play important roles in determining the levels of leptin in children and adolescent with type1 diabetes mellitus..

Keywords: leptin, insulin, type1 diabetes

Introduction:

Type 1 diabetes (T1DM) is an autoimmune disease resulting from T helper 1 (Th1)-mediated destruction of the pancreatic beta cells, leading to insulin deficiency.

The incidence of T1DM is increasing worldwide, particularly in younger age groups[1,2]. Leptin which has a dual nature as hormone and cytokine plays an important role in the regulation of food intake and body weight regulation, glucose homeostasis, and energy expenditure through hypothalamic pathways[3-5], it interacts with other hormonal mediators and regulators of energy and metabolism such as insulin, glucagon, growth hormone and glucocorticoids[6]. Moreover, it plays a crucial role in the inflammatory processes involving T-helper cells and modulating T-helper cell activity in the cellular immune response. Several studies have implicated leptin in the pathogenesis of autoimmune inflammatory condition[7,8].

T1DM is characterized by sever insulinopenia and dependence on exogenous insulin therapy to preserve life[9]. But the exact relationship between leptin and insulin is not clear and is sometimes controversial. Although insulin is secreted from the pancreatic beta cells rather than from adipocytes, the secretion of both hormones is influenced by overall amount of fat stores as well as by short-term changes in energy balance. Also, insulin receptors are located in the same key hypothalamic areas as leptin receptors. Insulin secretion is stimulated acutely in response to meals, whereas leptin secretion is not[10]. On the other hand, the incidence of diabetes is presumed to be due to unidentified environmental factors. One environmental factor that may have an effect on T1DM incidence is bodyweight, or factors associated with obesity. Several studies have suggested that a patient's body mass index (BMI) may play an important role in determining the disease[11,12].

The measurement of serum leptin level together with insulin (dosage, and concentration); as well as other metabolic control in type 1 diabetes (treated and newly diagnosed) have been rarely investigated. This study investigated leptin concentrations in children and adolescents with type1 DM before and after starting insulin therapy, also in a group of type1 diabetic who were on intensive insulin therapy, and in healthy control group. Furthermore, pursue whether or not leptin levels are related to metabolic control, lipid profile, and insulin dosage, duration of disease, BMI, age, and gender in type1 diabetes mellitus.

Patients and Methods:

A total of 77 subjects were examined in this study including 57 with type1 children and adolescents attending to the National Diabetic Center (NDC) - Al-Mustansiriya University.

Fifteen of them refers as **G(1)** (6 boys, 9 girls) a newly diagnosed patients who were studied before **G1(A)** and after **G1(B)** 6-7 days of insulin treatment, they had not yet received exogenous insulin therapy.

The other 42 patients refers as **G2** (19 boy, and 23 girl) previously diagnosed type 1 diabetic patients; they were on intensified insulin regimen with multiple daily insulin injection (2 injection of insulin mixture, NPH and regular insulin before breakfast and dinner for 2 years or more). A twenty persons age matched healthy subjects refers as **G3** serves as control group (8 boy, and 12 girl), they were taking no medication and displayed no evidence of impaired glucose tolerance or any other endocrine dysfunction that may be contribute to leptin dysregulation[13].

All subjects were matched with age, and sex. Weight and height were measured in indoor clothing without shoes, and the BMI was calculated as weight (kg)/height^2 (m^2).

Blood was drawn after overnight fasting for ~12 hours in non-diabetic subjects. Type I diabetic subjects were examined at least 2h after insulin injection and meal ingestion which should not affect serum leptin concentration[13].

Blood for baseline serum leptin levels in group1 G1 (A) was drawn at the time of diagnoses and before any therapy was initiated.

Laboratory evaluations consisted of measuring glycemic control including (fasting serum glucose (FSG), glycated hemoglobin HbA_{1c}), and lipid profile. Estimation of Leptin :The DRG Leptin Enzyme Immunoassay Kit (ELISA) provides materials for quantitative determination of leptin. As well as, this study determined the insulin dose/kg/d.

Hemoglobin A_{1c} program intended for the determination of Glycated hemoglobin ($A1_c$) in human depended on high performance liquid chromatography (HPLC) which was supplied by Variant Company, USA. Glucose level was determined using kits supplied by Randox, UK. Total cholesterol TC, Triglycerides were determined using kits from (biomaghreb, Sa, France).

Statistical analysis:

Data were analyzed using computer facility-the available statistical packages of SPSS-11 (statistical packages for social sciences-version 11.0).

Data were present in simple measures of Mean±SD. The significance of difference between quantitative variables was tested using student t-test for comparing between two means of independent groups. P value equal and less than 0.05 was used as the level of significance, and P value equal and less than 0.01 was used as the level of a highly significant. Pearson correlation coefficient is significant at the 0.05 level (2-tailed)

Results:

Table (1) compares the clinical characteristics of newly diagnosed type1 DM, diabetic patients on conventional insulin therapy, and controls group. They were similar with age, gender. It showed that newly diagnosed patients G1 before starting insulin G1(A) therapy had a significant lower leptin levels and higher FBG versus after (6-7) day of insulin therapy G1(B) (6.5 ± 1.52 vs. 7.95 ± 1.62; and 13.98 ± 2.96 vs. 11.5 ± 1.99) respectively, and there is no significant differences in the BMI between the two groups.

Moreover, after initiation with insulin therapy in the group1, the serum leptin levels become similar to the leptin levels in the control group. Also, they had a higher serum FBG, HbA_{1c} compared to control group.

On the other hand, the patients on conventional insulin therapy G2 for more than 2 years had higher serum leptin levels, insulin doses, BMI, and lower FBG, HbA_{1c} versus the newly diagnosed group G1. As well as, it showed that diabetic patients G2 had significantly higher serum leptin levels, BMI, FBG, and HbA1c versus healthy control G3. Also, there is no significant difference in the levels of TC, and TG among the studied group.

Table 2 showed the clinical characteristics data in children and adolescent according to the gender; it appears that serum leptin levels were significantly higher in diabetic girls versus boys (10.8 ± 2.42 ng/dl vs. 7.9 ± 2.3 ng/dl).

Although, there was a higher significant difference in BMI, and triglycerides level in diabetic girls compared to diabetic boys (17.7 ± 1.6 vs. 15.8 ± 1.1; 171.8 ± 23.5 vs. 156.3± 21.2) respectively. But, there is no significant difference in the insulin dosage between girls and boys.

(Table 3) showed that serum leptin levels had a significant positive correlation among BMI (r=0.727, P<0.01), insulin dose (0.822, P<0.01), and duration of diabetes (r=0.56, P<0.01), TG (r= 0.401, P< 0.40, P<0.05). Moreover, there is no significant correlation between leptin levels and age, FBG, HbA_{1c}, and TC.

On the other hand, there is a significant positive correlation between insulin dose and BMI (r= 0.62, P<0.01), and significant negative correlation between insulin dose and FBG (-0.41, P<0.05) but there is no significant correlation between insulin dose and HbA_{1c} in diabetic patients.

Table (1): the Clinical characteristics data of the studying group.

Clinical characteristics	G1: Newly diagnosed DM (n=15)		G2: type1 during insulin therapy >2y (n=42)	G3: Controls (no=20)
	G1(A):Baseline (0 day)	G1(B)After insulin treatment (6-7)		
Sex (boy: girl)	(6: 9)	(6: 9)	(19 : 23)	(8 : 12)
Age (years)	10.89 ± 2.6	10.89 ± 2.6	11.1±3.15	11.26±3.6
BMI (kg/m^2)	13.01 ± 2.01 $^{\alpha}$	13.2 ± 2.1 $^{\alpha}$	16.89 ± 4.05$^{\#}$	13.18 ± 4.42
Duration of DM (yrs)	----	----	4.9 ± 0.11	----
Serum leptin (ng/ml)	6.5 ± 1.52$^{*, \alpha}$	7.95 ± 1.62 $^{\alpha}$	9.34 ± 2.62 $^{\$}$	7.31 ± 1.53
FBG (mmol/l)	251.6 ± 40.2$^{*, \alpha, \$}$	200.1 ± 39.8 $^{\alpha, \$}$	162.8 ± 38.1$^{\$}$	88.2 ± 30.1
HbA_{1c}	9.01 ± 2.3 $^{\alpha, \$}$	8.98 ± 1.9 $^{\alpha, \$}$	7.30 ± 2.24 $^{\$}$	5.11 ± 0.61
Insulin (U/kg/d)	----	0.71 ± 0.1 $^{\alpha}$	0.9± 0.2	-----
Cholesterol (mg/dl)	189.7± 21.5	188.9 ± 19.8	189.7 ± 20.9	180.2 ± 18,9
Triglycerides (mg/dl) (mg/dl)	169.5 ± 23.7	161.1 ± 22.8	160.8 ± 22.0	158.9 ± 21.5

Data are mean ± SD, * P<0.05 G1(A) vs. G1(B); $^{\alpha}$ P<0.05 G1(A), G1(B) vs. G2 ; $^{\#}$ P<0.05 G2, G1(B) vs. G3; $^{\$}$ P<0.01 G1(A), G1(B), G2 vs. G3

Table (2): Clinical characteristics in diabetic patients G2 according to gender.

Clinical characteristics*	Boys (n:18)	Girls (n:24)
Age (years)	19	23
BMI (kg/m^2)	15.8 ± 1.1	17.7 ± 1.6*****
Duration of DM (yrs)	4.3 ± 2.2	3.8 ± 1.6
Serum leptin (ng/ml)	7.9 ± 2.3	10.8 ± 2.42*****
FBG (mmol/l)	8.9 ± 2.1	9.1 ± 2.3
HbA_{1c}	7.19 ±1.98	7.56 ± 2.1
Insulin (U/kg)	0.9 ± 0.19	0.91± 0.20
Cholesterol (mg/dl)	188.5 ± 20.5	189.9 ± 23.4
Triglycerides (mg/dl)	156.3± 21.2	171.8 ± 23.5*****

Data are mean ± SD, ***** P≤ 0.05 was considered significant.

Table (3): The correlation between serum leptin and variables in type1 DM.

Parameters	DM type1
	R
Leptin – age	0.287
Leptin – insulin	0.822**
Leptin – FSG	- 0.102
Leptin– HbA_{1c}	0.133
Leptin – BMI	0.727**
Leptin – Duration	0.56 **
Leptin – TC	0.20
Leptin – TG	0.401 *
Insulin – BMI	0.62 **
Insulin – HbA_{1c}	0.183
Insulin – FBG	- 0.41 *

*correlation is significant at the 0.05 level (2-tailed)
**correlation is a highly significant at the 0.01 level (2-tailed)

Discussion:

It is still controversial whether insulin modulating leptin levels or not. Some [14,15] studies found that insulin treatment is associated with increased leptin levels, but not in other[16]. Some studies[17,18] found that there is a strong positive correlation between fasting leptin levels and fasting insulin concentration. The present study revealed that a serum leptin level is low in patients with newly diagnosis diabetes. This results might be related directly to the absent or low insulin levels in these patients resulting from poor glycemic control, or might be related to the presence of lower amount of fat mass and body mass index[19,20].

However, the current study revealed significantly lower BMI in newly diagnosed patients versus

patients on intensive insulin therapy for more than two years. One in vivo study confirmed that *ob* mRNA and leptin levels decreased to barely detectable levels with fasting (low insulin status), but were restored to normal within 4hr after re feeding or administration of insulin[21,22].

On the other hand, nutritional depletion and patients' catabolic state could potentially explain the lower leptin levels. Some studies[23,24] showed that insulin deficiency might be associated with changes in serum protein binding and/or the binding of leptin in peripheral pools with subsequent enhancement of leptin clearance. The importance of leptin clearance over leptin production in determining the ambient serum leptin has been proven[23,25]. Moreover, even free fatty acids, which increase with insulin deficiency, bind to leptin and could potentially affect leptin clearance[26]

After the initiation of insulin therapy, leptin levels increases significantly, and become comparable to control group after 6-7 days of insulinization even before a significant change in BMI. These data were supported by the findings that low dose infusions of insulin can prevent the decrease of leptin during fasting in human with type1 and 2 diabetes[21].

These data support a direct relationship between the circulating insulin concentration and leptin secretion[21]. Wabitsoh *et.al*,[27] found that insulin stimulated leptin expression and synthesis in humans stems from vitro studies with differentiated human adiposity.

Some studies[17,18] introduce a molecular mechanism has been proposed to explain how insulin and nutritional signals might regulate serum leptin, the candidate transcription factor, adipocyte determination differentiation dependent factor1/sterol regulatory element binding protein1 (ADD1/SREBP)I which is activated by insulin and prior feeding causes increased leptin secretion and *ob* mRNA in fat tissue. Despite this, however, in *vitro* studies of cultured fat tissue and cells have yielded opposing result concerning the sustained effect of insulin on leptin secretion[28,29].

Another finding in this study is a positive correlation between leptin levels and diabetic duration in patients on intensive insulin therapy; Bideci, *etal*.,[30] suggested that the duration of insulin therapy might be responsible for this result.

The present study found that serum leptin levels in treated patients G2 were significantly higher than healthy control G3, and patients with newly diagnosed patients after (6-7) days of insulin therapy. This can be explained by the body composition of type1 DM is altered with the progressive use of multiple insulin injection in late puberty shifting body composition towards increased body fat mass, or might be related to increased exogenous insulin requirements during puberty to reduced insulin sensitivity[31]. So, higher insulin doses administered might lead to transient hyperinsulinemia and subsequent hyperleptinemia.

The present study showed a significant difference in the insulin dosage between patients on intensive insulin therapy and patients after (6-7) days of insulinization. Wauters *et al.* showed that leptin is important in weight regulation and acts to control food intake and energy expenditure. They found that leptin concentrations increased with obesity and tend to decrease with weight loss and correlate with insulin levels in patients with hyperinsulinemia.[32]

A putative regulation of leptin expression by glucose is conflicting. In mice and humans *ob* mRNA was inhibited by food restriction associated with glucopenia and was stimulated by injection of glucose in some[33,34], but not in others[35]. The present study showed that there is no significant correlation between serum leptin levels and fasting blood glucose (FBG) in type1 DM patients receiving insulin this might be due to related leptin hormone to fat levels more than glucose levels. Otherwise, a very high glucose levels in patients with newly diagnosed type1 DM prior the blood sampling might also suppress leptin levels. But this can be explained by the newly diagnosed patients in the present study had lower BMI compared to treated diabetic patients. Thus, a reduced body fat mass due to increased lipolysis following insulin deficiency might have resulted in reduced absolute leptin in these patients. Also, the current study showed a significant positive correlation between leptin level to BMI, and showed that BMI increased significantly in group {G(A2)} treated diabetic patients. Kiess *etal.*[36] found that BMI increases with insulin dose and the number of insulin injection per day; however, the current study showed a significant positive correlation between insulin dose and BMI.

Another correlation found in the current study was a positive correlation between serum leptin levels and serum triglycerides levels in treated diabetic patients (table 3), this may be indicate to the interaction of serum leptin levels with lipid metabolism.

The current study reveals gender difference in leptin levels. It was found that girls have significantly higher leptin levels than boys. Studies[37,38] suggested that raised leptin levels were related to insulin dose. However, in the current study, daily insulin doses were not different between girls and boys. The most likely explanation for this discrepancy is that in girls the increase in BMI during and after puberty is mainly associated with an increase in adipose tissue, whereas in boys the increase in BMI is due to an increase in muscle mass. Besides this sex difference in body composition direct influences of sex steroids may be causative[39]. Garcia-Mayor *etal*,[40] reported that the leptin was the first hormone to rise following by FSH and later by LH and estradiol. A similar pattern

occurred in boys, despite the fact that leptin dropped after 10 yrs when testosterone rises. In girls the four hormones rose progressively of period from prepuberty to overt puberty. In boys, leptin decreased from prepuberty to overt puberty, while FSH, LH, and testosterone rose.

This study conclude that insulin therapy is a potent stimulator of serum leptin production in newly diagnosed type1 diabetes patients, and the direct effects of insulin on leptin levels have largely supported a positive correlation between the two hormones. Although, the relation of serum leptin together with assessment of other risk factors could help to identify the development of hyperglycemic process.

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